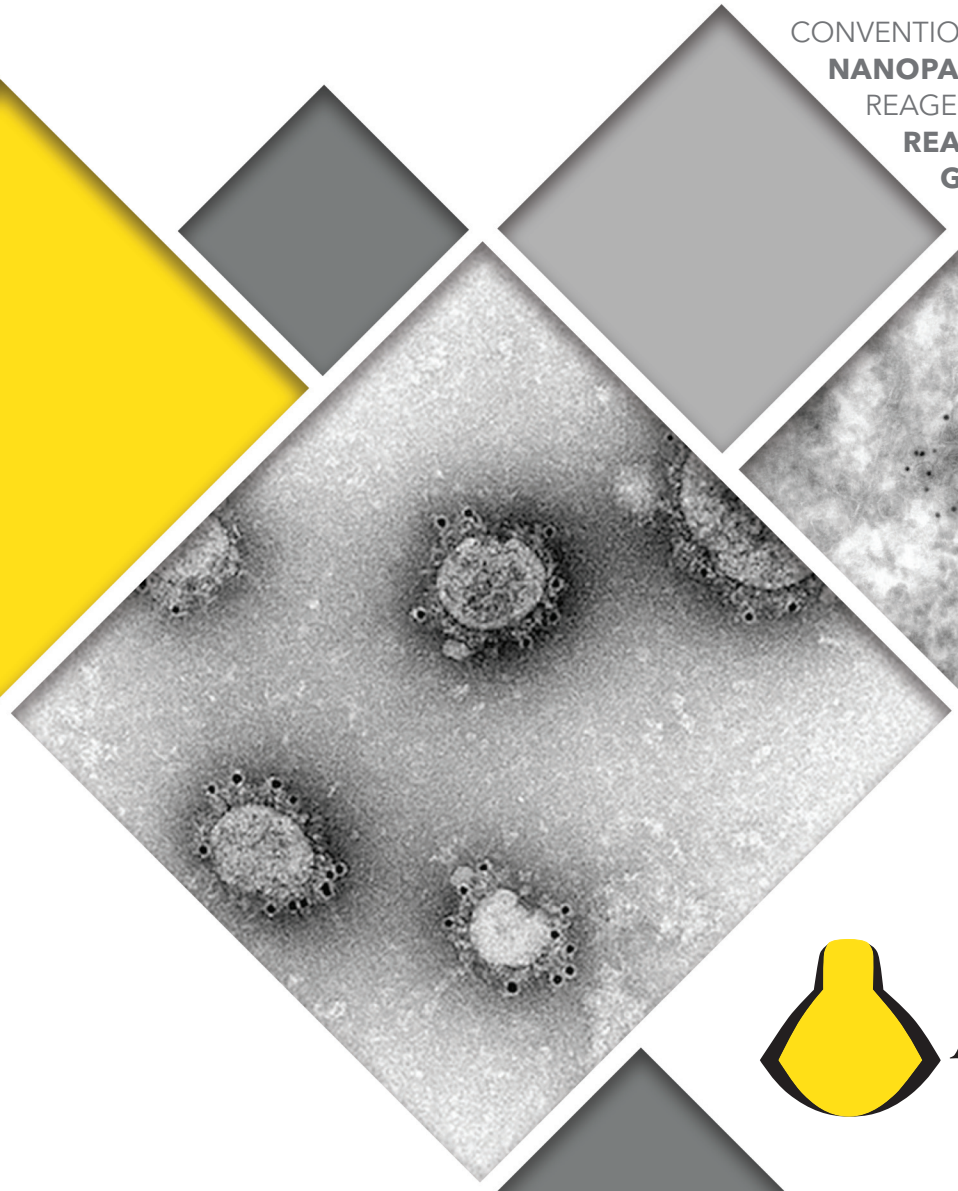
AURION **IMMUNOGOLD REAGENTS**

EDITION III

CONVENTIONAL IMMUNOGOLD REAGENTS, **GOLD NANOPARTICLES,** ULTRA-SMALL IMMUNOGOLD REAGENTS, **R-GENT SILVER ENHANCEMENT REAGENTS,** GOLD TRACERS, **COLLOIDAL GOLD BASED PROTEIN STAIN,** IMMUNOGOLD REAGENT KITS, **TECHNICAL INFORMATION,** AND MORE...



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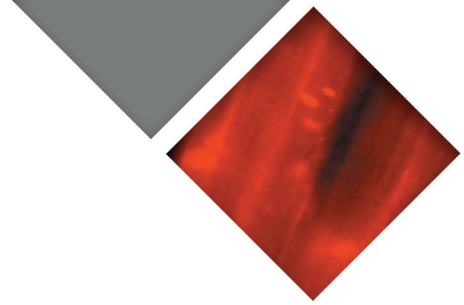

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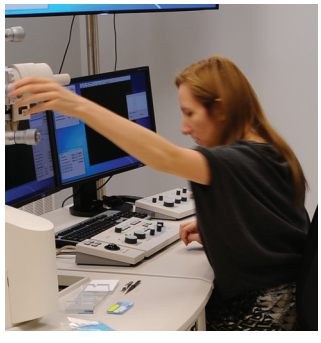

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AURION **IMMUNOGOLD REAGENTS**

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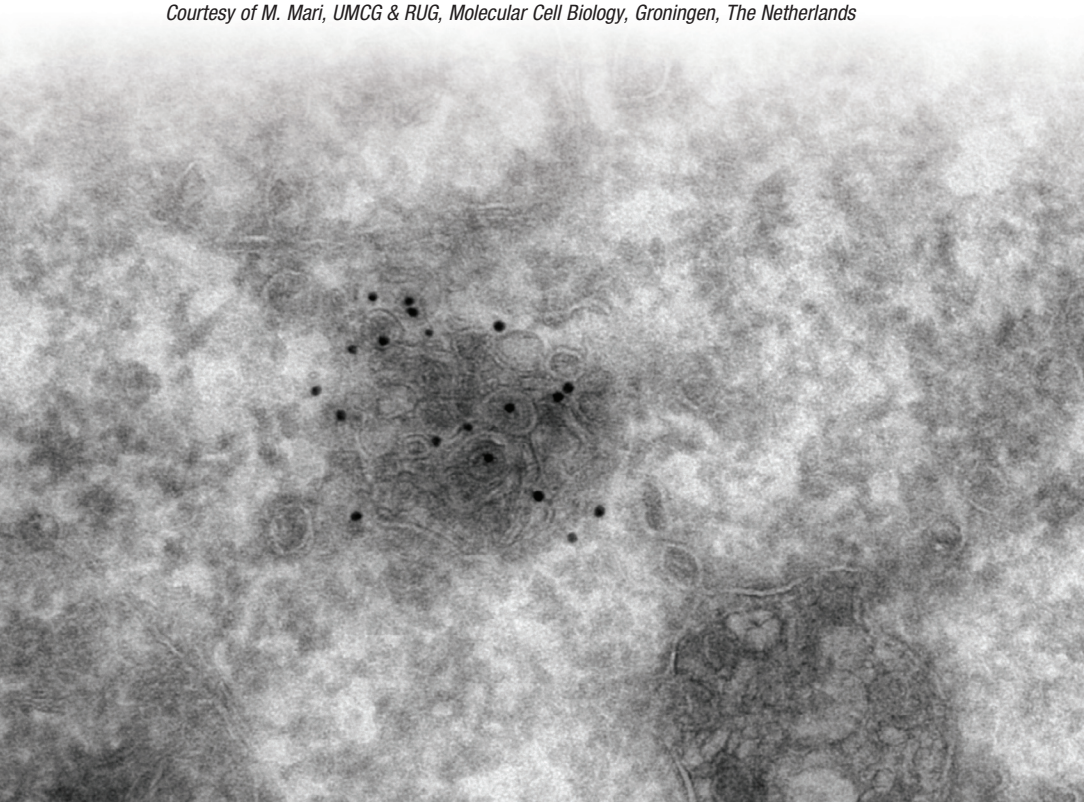
Courtesy of M. Mari, UMCG & RUG, Molecular Cell Biology, Groningen, The Netherlands



AURION

NEW SERIES...

2nm Gold Nanoparticle Conjugated Goat-anti-Rabbit IgG and Goat-anti-Mouse IgG

Fill the gap between ultra-small gold reagents and the conventional sized 6-25nm gold conjugates.

See page 3.

Gold Nanoparticles – Carboxyl Functionalized

Facilitate conjugating gold nanoparticles to molecules that cannot be conjugated via the classic direct adsorption method.

See page 11.

Electron Microscopy Sciences

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PRODUCT INFORMATION

III Conventional ImmunoGold Reagents

Conventional Immunogold Reagents are available in four size classes. The monodisperse size population makes the conjugates suited for multiple labeling with no overlap. The Conventional Immunogold Reagents are the classical conjugates in immuno electron microscopy; they are a good choice when the antigen is abundant and the accessibility of the antigen is relatively good.

Introduction

The conventional labeling approach in transmission and scanning electron microscopy utilizes secondary immunogold reagents based on particles that can be observed without enhancement. These conjugates are suited for single and multiple labeling in electron microscopy, when the number of antigens available for binding is such that a relevant signal can be obtained.

The AURION Conventional Immunogold Reagents are built around colloidal gold particles with sizes of 6, 10, 15 or 25 nm. The particle population is monodisperse and thus shows minimal size variation and overlap. Typically, the coefficient of variance for the 6 and 25 nm particle size conjugates is less than 12%, whereas the 10 and 15 nm size conjugates show less then 10% variation.

The table below lists a few physical characteristics of gold conjugates.

Particle Diameter	+/– #Au atoms	+/– MWt. (daltons)	+/– #Particles /ml	+/– #Ab (/part)
6 nm	6500/td>	1.3·106	$2.4 \cdot 10^{13}$	1-2
10 nm	30·103	6·106	$5 \cdot 10^{12}$	7-12
15 nm	100·103	20·106	$1.5 \cdot 10^{12}$	25-40
25 nm	470·103	92·106	$3.3 \cdot 10^{11}$	115-180

Features

- **For single and multiple labeling**
- **Gold particle sizes of 6, 10,15 and 25 nm**
- **Monodisperse particle population**
- **OD_{520} nm of 1.0 to warrant cluster free storage using a dot-spot test system.**
- **Minimal size variation and overlap**
- **Coefficient of variance: <12% for the 6 and 25 nm conjugates and <10% for the 10 and 15nm conjugates**

Product Description

AURION Conventional Immunogold Reagents are tailored to contain 10-20 µg of specific protein/ml. The reagents are supplied in PBS with 1% Bovine Serum Albumin and 15 mM NaN_3 at an OD_{520}nm of 1.0 to warrant prolonged cluster free storage. The activity of each lot is determined using a dot-spot test system as described by Moeremans et al., J. Immunol. Methods, 74, (1984), 353. Actual lot specifications (size, variation and expiry date) are indicated on the accompanying package insert.

Regular package: for the labeling of 1000-2000 grids

Small package: for the labeling of 400-800 grids

Specificity

Aurion offers the widest range of Conventional Immunogold Reagents.

AURION Conventional Immunogold Reagents are prepared using the highest quality antibodies or binding agents available. All antibodies are immuno affinity purified and immuno cross-adsorbed to reduce non-specific reactions.

Storage

AURION Conventional Immunogold Reagents have a guaranteed shelf life of 18 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.

TECHNICAL TIPS...

Application Instructions

Detailed information is provided on the package inserts.

On-Grid Labeling

For most applications grids are floated on top of drops of dilute reagent displayed on a sheet of Parafilm™. The use of gold or nickel grids is recommended.

Whole mount and intact cell labeling

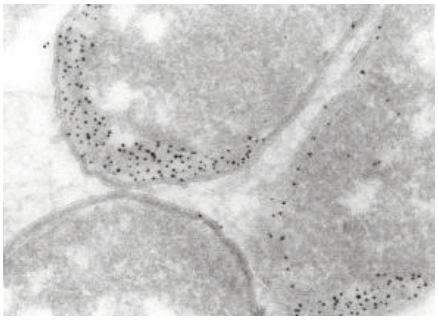
Specimens are kept floating in dilute reagent on a rocking table.

Recommended Incubation Solution:

PBS,
(10 mM Phosphate buffer, 150 mM NaCl),
0.1-0.2 % AURION BSA-C™
15 mM NaN_3
pH 7.4

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections



Immunolabeling of the periplasmic space in ultrathin cryosections of Escherichia coli with a protein A gold conjugate. Courtesy M. de Jong

III 2nm Gold Nanoparticle Conjugated Goat-anti-Rabbit IgG and Goat-anti-Mouse IgG

This new series of immunogold reagents have the capacity to fill the gap between Aurion's unique ultra-small gold reagents and the conventional sized 6-25nm gold conjugates.

Features

- Monodisperse size population
- Coefficient of variance < 15%
- For pre- and post-embedding labeling
- Direct visualization in EM optional
- Homogeneous silver enhancement
- In EM applications; use R-Gent SE-EM
- For LM/bio-assays use R-Gent SE-LM

Product Description

Aurion 2nm conjugated GAR and GAM IgG are prepared using a monodisperse size population of 2nm gold nanoparticles. Due to its small gold nanoparticle size, labeling density with these Aurion 2nm Immuno Gold Reagents is significantly higher compared to particle density obtained with comparable Conventional Immuno Gold Reagents. This makes these 2nm immunogold conjugates especially suited for detection of low copy numbers of antigen.

Aurion 2nm Immunogold Reagents contain 60-80 µg of specific protein/ml for IgG conjugates. The average gold particle diameter is 2nm.

The reagents are supplied in PBS with 1% Bovine Serum Albumin and 15 mM NaN_3. The activity of each lot is determined using a dot-spot test system as described by Moeremans et al., J. Immunol. Methods, 74, (1984), 353.

The products are available in two package sizes:

0.4 ml for 320 grids or 160 slides

1.0 ml suited for 800 grids or 400 slides

Specificity

AURION 2nm Immunogold Reagents are prepared using the highest quality antibodies or binding agents available. Antibodies are immuno affinity purified and immuno cross-adsorbed to reduce non-specific reactions.

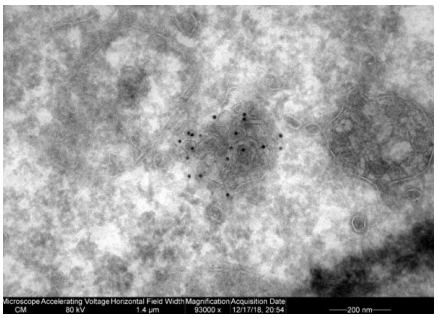
Conjugated to Goat anti Rabbit IgG (H&L) and Goat anti Mouse IgG (H&L)

For other species and specificities we kindly refer you to our Custom Labeling Service.

Storage

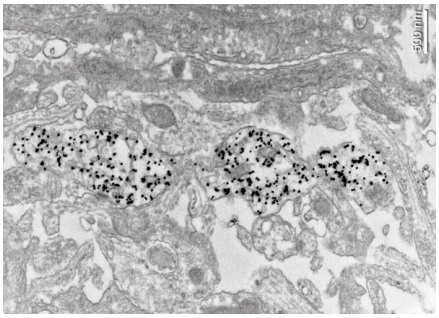
AURION 2nm Immuno Gold Reagents have a guaranteed shelf life of 18 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.



Post embedding immunolabeling: Detection of Lamp2 on Tokuyasu sections of 4% PFA fixed HeLa cells using AURION GAM IgG 2nm and AURION R-Gent SE-EM.

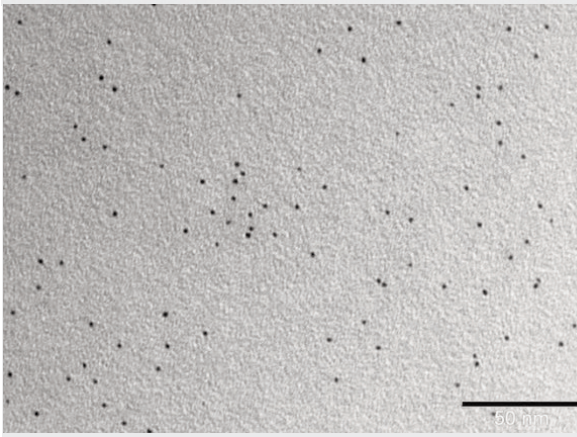
Courtesy of M. Mari, UMCG & RUG, Molecular Cell Biology, Groningen, The Netherlands



Pre-embedding labeling on 2%PFA/GA fixed and freeze thaw permeabilized organotypic cultured brain slices using rabbit anti GFP, AURION GAR 2nm and R-Gent SE-EM.

Courtesy of Y. Sun, D. Guerrero-Given and N. Kamasawa; EM Core Facility, Max Planck Florida, Institute for Neuroscience, Jupiter, FL USA

NEW SERIES



Typical size distribution of AURION 2nm Immuno Gold particles

TECHNICAL TIPS...

Application Instructions

Detailed information is provided on the package inserts.

On-Grid Labeling

For most applications grids are floated on top of drops of dilute reagent displayed on a sheet of Parafilm™. The use of gold or nickel grids is recommended.

Whole mount and intact cell labeling

Specimens are kept floating in dilute reagent on a rocking table.

Recommended Incubation Solution:

PBS,
(10 mM Phosphate buffer, 150 mM NaCl),
0.1-0.2 % AURION BSA-C™
15 mM NaN_3
pH 7.4

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections

PRODUCT INFORMATION

AURION

TECHNICAL TIPS...

Application Instructions

Electron Microscopy Labeling

For post-embedding labeling, incubate thin sections by placing grids on drops of reagent arrayed on a sheet of Parafilm™. The use of nickel grids is recommended, especially if silver enhancement procedures are intended.

For pre-embedding labeling, float specimens in dilute reagent on a rocking table.

For more information on post and pre-embedding labeling;

- *Post-embedding Immuno Incubation Protocol*
- *Pre-embedding Immuno Incubation Protocol*
- *Ultra Small Gold Reagents and R-Gent SE-EM in Pre-embedding single and double labeling*

Light Microscopy Slide or Coverslip Labeling

Apply a few drops of dilute reagent to cover the specimen.

Recommended Incubation Solution:

PBS,
(10 mM Phosphate buffer, 150 mM NaCl)
0.1-0.2 % AURION BSA-c™
15 mM NaN_3
pH 7.4

Additional Information

"Ultra Small Gold Reagents and R-Gent SE-EM in Pre-embedding Single and Double Labeling" Hong Yi and Jan L.M. Leunissen

"Optimized Immuno Labeling using AURION BSA-c™ and AURION Blocking Solutions" by J.L.M. Leunissen and P.F.E.M. van de Plas

For additional information please see the Aurion Immunogold Newsletter and Protocol sections.

III Ultra Small ImmunoGold Reagents

Aurion Ultra Small Immunogold Reagents are prepared with subnanometer gold particles. These particles have far less influence on the adsorbed antibodies or detecting molecules, and consequently the conjugates behave as though they are uncoupled. In conjunction with the highly efficient and easy-to-use R-Gent SE-LM and SE-EM silver enhancement reagents, the Ultra Small Immunogold Reagents are the best choice for any application.

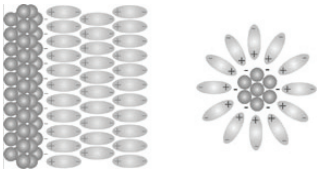
Introduction

Reduction of the gold particle size provides Ultra Small Immunogold Reagents with fundamentally different characteristics when compared with conjugates built around larger particles. While the Conventional Immunogold Reagents can be thought of as particles coated with proteins, Ultra Small Immunogold Reagents are proteins coated with one or more gold particles. With this structure, both the overall size of the conjugates, as well as steric hindrance are decreased.

The table below lists a few physical characteristics of gold conjugates.

Particle Diameter	+/– #Au atoms	+/– MWt. (daltons)	+/– #Particles /ml	+/– #Ab (/part)
0.8 nm	15	$3{\cdot}10^{3}$	$5{\cdot}10^{15}$	0.1-1?
6.0 nm	6500	$1.3{\cdot}10^{6}$	$2.4{\cdot}10^{13}$	1-2
10 nm	0·103	$6{\cdot}10^{6}$	$5{\cdot}10^{12}$	7-12

Aurion offers Ultra Small Immunogold Reagents with an average gold particle diameter of 0.8 nm or less. These ultra small gold particles can be visualized directly in high angle annular dark-field-scanning TEM. However, the gold signal is normally visualized after increasing the particle diameter with silver enhancement. The reagents can be used in electron and light microscopy as well as in blotting experiments. The universal applicability makes it easy to compare results obtained with different procedures.



The small gold particles also have a tight particle surface curvature which makes it less likely that a structured water dipole layer will build-up around the gold particles. Hence, the hydrodynamic radius of the ultra small gold colloids is reduced. Finally, small gold particles carry less net negative charge; thus, they undergo less charge determined repulsion when approaching the sample surface.

Product Description

AURION Ultra Small Immunogold Reagents contain 60-80 µg of specific protein/ml for IgG conjugates. F(ab')2, Fab and biotinylated albumin conjugates contain equimolar amounts of conjugated protein. The average gold cluster diameter is less than 0.8 nm.

AURION Ultra Small Immunogold Reagents are used in conjunction with AURION R-Gent SE-EM or SE-LM silver enhancement reagents, developed for electron microscopy and light microscopy/immunoblotting respectively.

The reagents are supplied in PBS with 1% Bovine Serum Albumin and 15 mM NaN_3.

The activity of each lot is determined using a dot-spot test system as described by Moeremans et al., J. Immunol. Methods, 74, (1984), 353.

The products are available in two package sizes: *Regular package:* for 1200 grids or 600 slides, *Small package:* for 480 grids or 240 slides.

Specificity

Aurion offers the widest range of Ultra Small Immunogold Reagents. The most commonly used reagent types are available as intact IgG, F(ab')2 and Single Fab conjugates. To view the complete list of available reagents click here.

AURION Ultra Small Immunogold Reagents are prepared using the highest quality antibodies or binding agents available. All antibodies are immuno affinity purified and immuno cross-adsorbed to reduce non-specific interactions.

Storage

AURION Immunogold Reagents have a guaranteed shelf life of 18 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.

III Blocking Solutions

The signal-to-noise ratio determines the quality of any detection experiment. This ratio is in principle determined by the characteristics of specimen and detection reagents. With adequate blocking and incubation media, background reactions are minimized whereas specific reactions are not hampered.

Aurion has developed Blocking Solutions which effectively block 'sticky' surfaces by multi-point hydrophobic and charge-based interactions. The Blocking Solutions are specifically designed to meet the characteristics of each type of secondary conjugate.

Introduction

The AURION Blocking Solutions are used to prevent immunoreagents from binding non-specifically to specimens with "sticky" surface properties.

Procedures to eliminate background comprise three main steps:

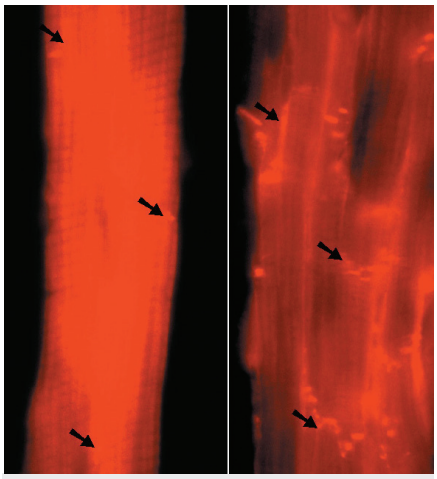
- *To suppress residual aldehyde activity*
- *To saturate multipoint hydrophobic moieties and positive charges with high molecular weight compounds such as those present in the AURION Blocking Solutions*
- *To reduce aspecific binding of immunoreagents caused by hydrophilic interaction with competing molecules in the incubation and washing solution. AURION BSA-c™ is a particularly effective reagent for this purpose.*

These steps should be balanced for optimum results.

Product Description

AURION Blocking Solutions are prepared using specially selected compounds. All ruminant proteins are obtained from healthy livestock.

AURION Blocking solutions contain Bovine Serum Albumin and Cold Water Fish Skin Gelatine in phosphate buffered saline with sodium azide as preservative. Normal serum may have been added as indicated on the label. The blocking capacity of each lot is determined using a dot-spot test system as described by Moeremans et al., J. Immunol. Methods 74, (1984), 353.



N-Cadherin detection in heart muscles

Immunofluorescence using Alexa 568 labeled Fab goat antimouse. For reasons of comparability areas of specific labeling are pictured with similar density (arrowheads).

Left hand panel: Background using a commonly used protocol obscures sites of specific labeling.

Right hand panel: N-Cadherin immunolabelled area obtained using Aurion Blocking Solution and BSA-c™ stand out with much clearer definition.

Courtesy of Lauren Hruby and John Harris; Dept. Physiology, University of Otago, Dunedin, New Zealand.

Each package contains 30 ml of solution. It accommodates 300 specimens for light microscopy at 100 µl/specimen (~ 3 drops), or 1000 EM grids at 30 µl/specimen (~ 1 drop).

Specificity

Blocking Solutions are available in the following specificities:

serum-free: for use with Protein A and Protein G Gold conjugates.

with Normal Goat serum: for use with reagents based on secondary antibodies raised in Goat.

with Normal Rabbit serum: for use with reagents based on secondary antibodies raised in Rabbit.

with Normal Sheep serum: for use with reagents based on secondary antibodies raised in Sheep.

with Normal Donkey serum: for use with reagents based on secondary antibodies raised in Donkey.

Storage

AURION Blocking Solutions have a guaranteed shelf life of 18 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.

TECHNICAL TIPS...

Application Instructions

Detailed information is provided on the package inserts.

Electron Microscopy

On-Grid Labeling

Placing grids on top of drops of Blocking Solution arrayed on a sheet of Parafilm™ after they have been treated for aldehyde inactivation.

Pre-embedding Labeling

Floating specimens in Blocking Solution, preferably on a rocking table.

Light Microscopy

On-slide or -coverslip Labeling

Applying a few drops of Blocking Solution to cover the specimen.

- Bio Assays

Incubating specimens (e.g. immunoblot strips) in Blocking Solution on a rocking table in screw-cap sealed disposable tubes.

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections .

PRODUCT INFORMATION

TECHNICAL TIPS...

Application Instructions

Detailed information is provided in the package inserts.

Recommended Incubation Solution:

PBS,
(10 mM Phosphate buffer, 150 mM NaCl)
0.1-0.2 % AURION BSA-c™
15 mM NaN_3
pH 7.4

After addition of the BSA-c™ concentrate the pH should be checked and adjusted to 7.4 if necessary.

This Incubation Solution is used throughout the whole set of reactions and washing steps (i.e. from immediately after the blocking step, through the primary antibody incubations, in-between washing steps, secondary incubation and the final washing steps).

Additional Information

"Optimized Immuno labeling using AURION BSA-c™ and AURION Blocking Solutions" by J.L.M. Leunissen and P.F.E.M. van de Plas

For additional information please see the Aurion Immunogold Newsletter and Protocol sections

III Incubation Solution Additive AURION BSA-c™

In the blocking step, hydrophobic moieties causing "stickiness" in the specimen surface are rendered hydrophilic to minimize background. Nevertheless, the more dynamic charge-based interaction between the specimen surface and immunoreagents also needs to be controlled in order to eliminate background.

Aurion has developed BSA-c™, a unique incubation buffer additive with an unparalleled ability to effectively prevent charge based background. BSA-c™ is prepared by acetylation of bovine serum albumin (BSA). Polycationic sites in the specimen interact readily with negatively charged acetylated BSA molecules. This significantly reduces the risk that such sites might bind negatively-charged immunoreagents and immunogold conjugates and thus reduces the risk of background.

Introduction

Procedures to eliminate background comprise three main steps:

- *To suppress residual aldehyde activity*
- *To saturate multipoint hydrophobic moieties and positive charges with high molecular weight compounds such as those present in the AURION Blocking Solutions*
- *To reduce aspecific binding of immunoreagents caused by hydrophilic interaction with competing molecules in the incubation and washing solution.*

AURION BSA-c™ is a particularly effective reagent for this purpose.

AURION BSA-c™ is a buffer additive that helps prevent immunodetection reagents (i.e. primary antibodies and secondary reagents) from binding nonspecifically to charged moieties within the specimen. Thus, it suppresses background competitively with little or no effect on the specific reaction. Its successful application is not limited to immunogold detections but it is equally efficient in fluorescent and enzyme-based detection systems. AURION BSA-c™ concentrations as low as 0.01-0.1% inhibit binding of gold conjugate to polycationic poly-l-lysine coated grids almost completely (>99%).

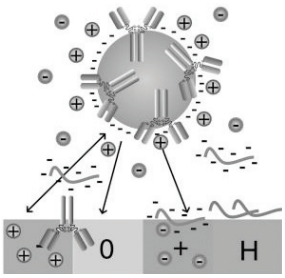
The surface properties of the specimen can be simplified by division into four compartments:

- negatively charged (polyanions, proteins, especially after aldehyde fixation, lipids);

0 neutral;

+ positively charged (histone proteins, polycations) and

H hydrophobic (lipids, fat droplets, resins). After an appropriate blocking step these areas are covered with blocking compounds.



In low ionic strength media negatively charged antibodies and gold conjugates are repulsed by negatively charged specimen areas which frequently may contain the antigens to be detected. Background does not likely occur in such areas. The positively charged areas attract antibodies and gold conjugates potentially leading to background. In a moderate ionic strength incubation solution, repulsion and attraction are diminished due to the presence of ions. The negatively charged BSA-c™ competes with the negatively charged antibodies and gold reagents for non-specific binding to the positively charged specimen compounds, thus reducing background to the greatest possible extent without interfering with antigen detection.

Product Description

AURION BSA-c™ concentrated solution contains acetylated bovine serum albumin as the functional constituent. By acetylation of amines on basic amino acids these groups are no longer as easily protonated and the isoelectric point of such molecules is lowered and hydrophobicity is increased. BSA-c™ is a 10% solution of acetylated BSA at slightly alkaline pH with Kathon CG as preservative. The bovine serum albumin that Aurion uses to prepare BSA-c™ is obtained from healthy livestock.

The charge dependent background inhibition capacity of the BSA-c™ in each lot is determined using a dot-spot test system with polycationic compounds.

Two package volumes are available which yield 1.5 - 3 and 5 -10 litres of incubation buffer respectively

Storage

AURION BSA-c™ concentrate has a guaranteed shelf life of 18 months from the date of quality control analysis. The products should be stored at 4-8°C. Freezing is not recommended.

III BSA/CWFS/Tween 20/Normal Sera

Many compounds have been added empirically to immunolabeling solutions for the purpose of minimizing background staining. Based on years of experience and controlled testing, Aurion has selected a group of compounds that are proven to be the most effective in their background reduction action. Researchers can obtain these items separately from Aurion and prepare blocking and incubation media to suit the needs of their own protocols.

Introduction

The ready-to-use Blocking Solutions and the incubation media additive BSA-c™ are tuned for optimum background prevention and signal-to-noise ratio. In-depth information can be found in the respective product data sheets.

AURION also offers a number of components that allow researchers to formulate blocking, incubation and wash solutions according to the needs of their own experiments.

Product Description

Aurion's Bovine Serum Albumin is obtained from healthy livestock. BSA should be dissolved in an appropriate buffer, such as phosphate buffered saline, taking care not to denature the protein by foaming. The addition of BSA may cause a drop in pH of the final solution and correction may be required. As a preservative the use of NaN_3 or Kathon CG is recommended.

The use of Cold Water Fish Skin Gelatin to prevent background reactions has been recommended by e.g. Behnke et al. (J. Cell Biol. 41, [1986], 386). The product is supplied as a liquid concentrate (40%).

Tween-20™ is a non-ionic detergent with a molecular weight of about 600 and a critical micelle concentration (CMC) of 0.06-0.07% in water at room temperature. Its working mechanism may in part be based on its action as a detergent, binding to the hydrophobic moieties of water insoluble compounds, rendering them hydrophilic. In addition, immuno-compounds may become incorporated into micelles when the Tween-20™ concentration is higher than the CMC, for instance at 0.1 % in PBS at pH 7.4.

Normal sera are used to counteract the non-specific interaction between the sample and immunoglobulins. They can be added to the blocking solution and the incubation media.

As a rule the normal serum species should be the same as the secondary antibody species (e.g. use normal goat serum with goat-anti-rabbit conjugates).

Note: normal sera should not be used in combination with Protein A and Protein G gold reagents.

Storage

The products should be stored at 4-8°C. Freezing is not recommended.

TECHNICAL TIPS...

Application Instructions

The following recipes have been tried succesfully by researchers in the field and by Aurion:

Blocking Solution for protein A or G reagents

Phosphate buffered saline
(10mM Phosphate buffer, 150 mM NaCl)
5 % BSA
0.1% CWFS Gelatin
15 mM NaN_3
pH 7.4

Blocking Solution for secondary antibody reagents

Phosphate buffered saline
(10mM Phosphate buffer, 150 mM NaCl)
5 % BSA
0.1% CWFS Gelatin
5-10 % normal serum
(same species as in the secondary antibody reagent)
15 mM NaN_3
pH 7.4

Incubation solution for Conventional Immunogold Reagents

Phosphate buffered saline
(10mM Phosphate buffer, 150 mM NaCl)
0.1-0.2 % BSA
0.1% CWFS Gelatin
1-5 % normal serum
(same species as in the secondary antibody reagent)
15 mM NaN_3
pH 7.4

Incubation solution for Ultra Small Immunogold Reagents

Phosphate buffered saline
(10mM Phosphate buffer, 150 mM NaCl)
0.8 % BSA
0.1% CWFS Gelatin
1-5 % normal serum
(same species as in the secondary antibody reagent)
15 mM NaN_3
pH 7.4

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections

PRODUCT INFORMATION

AURION

TECHNICAL TIPS...

Application Instructions

Detailed information is provided in the package inserts.

Electron Microscopy

Post-embedding enhancement (on-grid)

Place grids on drops of the enhancement mixture arrayed on a sheet of Parafilm™.

Pre-embedding enhancement

Incubate specimens (e.g. vibratome sections) in Petri dishes or Eppendorf tubes containing the enhancement mixture, with agitation. When enhancement is completed, the specimens are washed with distilled water. A fixation step with photographic fixer is not required.

Light Microscopy

On-slide or -coverslip Labeling

A few drops of the freshly prepared enhancement mixture are applied to cover the specimen. During enhancement the specimens are kept in a moist chamber. The ongoing enhancement may at intervals be monitored with an inverted light microscope. When enhancement is judged to be complete, the specimens are washed with distilled water. A fixation step with photographic fixer is not required.

Whole Mount Labeling

Specimens like floating (vibratome) sections can be enhanced in Petri dishes or 6-24 well plates.

Bio Assays

Depending on the type of assay the enhancement for bio assays can be performed in sealed plastic bags, Petri dishes or in disposable screw cap sealed tubes.

Storage

The AURION R-Gent SE-EM components are stored at 4°C and allowed to reach room temperature before use. SE-EM INITIATOR may be stored at -20°C for prolonged shelf life.

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections

III R-Gent SE-EM Silver Enhancement Reagents

For most applications the detection of ultra small gold particles in electron microscopy requires a particle enhancement procedure. Danscher's method has always been the standard for this purpose and for a long time attempts to improve the features of Danscher's system met with only limited success.

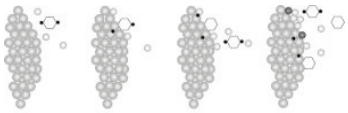
The goal in the development of AURION R-Gent SE-EM was to create a new system with enhancement efficiency and homogeneity at least comparable to Danscher's method, but with reduced acidity and light sensitivity. In addition, the reagents should have low viscosity for the suitability in pre-embedding immunogold labeling.

Introduction

The silver enhancement reaction is a gold particle-catalyzed reduction in which silver ions are reduced to metallic silver with a photographic developing compound as the electron source. In addition in many applications a "protective colloid" is added to the enhancement solution to limit diffusion of reagents to the gold particle surface, thus providing a means for controlling particle growth.

Product Description

AURION R-Gent SE-EM is a high efficiency silver enhancement reagent for electron microscopy. It intensifies the gold particles by homogeneous deposition of metallic silver on the particle surface. The resulting electron dense signal is easily detected and is compatible with heavy metal counterstaining. The reagent has extremely delayed auto-nucleation and can be used under standard laboratory light conditions. It also has low viscosity, which is especially advantageous for pre-embedding immunogold applications. The enhancement mixture has a pH of 8.1-8.2.

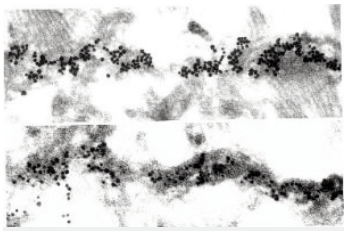


"...silver enhancement reaction is a gold particle-catalyzed reduction in which silver ions are reduced to metallic silver with a photographic developing compound as the electron source..."

AURION R-Gent SE-EM has been tested ntensively for the enhancement of AURION Ultra Small Immunogold reagents.

AURION R-Gent SE-EM is available in a kit containing the following:

- *30 ml or 90 ml of ready-to-use ENHANCER,*
- *3 ml of concentrated INITIATOR and*
- *30 ml of ACTIVATOR.*
- *a 3 ml empty dropping bottle, labeled "DEVELOPER".*



Desmin labeling in heart muscle with Ultra Small Gold conjugate and AURION R-Gent SE-EM. Courtesy Prof. Dr. Müller Höcker, University Munich, FRG.

The INITIATOR is a concentrated solution which must be diluted and activated before use, using the ACTIVATOR. This resulting mixture is the DEVELOPER.

The now ready-to-use DEVELOPER has a shelf life of one month. It is suggested to prepare fresh DEVELOPER at least every month.

The undiluted solutions have a shelf life of 10 months when stored at 4°C. INITIATOR can be stored at -20°C for prolonged shelf life.

For enhancement one drop of DEVELOPER is mixed with 20 drops of ENHANCER. The typical enhancement time is between 20 and 40 minutes. Specimens may be contrasted according to standard procedures.

The kit with 30 ml of ENHANCER accomodates up to 1000 EM grid specimens. The kit with 90 ml of ENHANCER is suitable for pre-embedding immunogold labeling; the number of specimens it accomodates depends on the volume of reagent used per specimen.

For each lot, the specific enhancement activity and level of autonucleation are monitored by spectrophotometric techniques.

III ECS – Enhancement Conditioning Solution

Aurion's Enhancement Conditioning Solution (ECS) is a concentrate that requires a 10x dilution with distilled grade water before use. It helps optimise enhancement by conditioning specimens labeled with (ultra small) gold conjugates before the silver enhancement procedure using Aurion R-Gent SE-EM. Aurion ECS is a prerequisite to condition enhanced specimens for a second incubation series in double labeling experiments (Yi et al, 2001, Journal of Histochemistry and Cytochemistry 49(3): 279-283).

Introduction

The silver enhancement reaction is a gold particle-catalyzed reduction in which silver ions are reduced to metallic silver with a photographic developing compound as the electron source. In addition in many applications a "protective colloid" is added to the enhancement solution to limit diffusion of reagents to the gold particle surface, thus providing a means for controlling particle growth.

Product Description

Incubation buffers suited for immuno gold labeling commonly contain ions such as phosphate and chloride which are not compatible with silver ions causing precipitation of insoluble silver salts. These ions therefore need to be thoroughly removed before enhancement. Whereas pure water is suited to fulfil this requirement in the purely chemical sense, it is rarely the best way when working with delicate specimens potentially affecting ultrastructural details vulnerable to drastic changes in ionic concentration. Using water it is also necessary to carefully blot the specimens and remove the water without drying before applying Aurion R-Gent SE-EM. Failing to do so will result in dilution and diminished activity of the silver enhancement reagents, especially when working with small volumes.

Aurion developed Aurion ECS to negotiate optimum conditions for enhancement while at the same time avoiding the risks posed by water. Its ionic strength is similar to immuno incubation buffers and its make up is compatible both with these buffers as well as with Aurion R-Gent SE-EM.

Aurion ECS is therefore highly recommended for single labeling experiments. It is an absolute requirement for double labeling where ECS not only reconditions specimens for the next immuno steps but also avoids the use of aldehyde fixation after the first immuno gold silver labeling sequence.

TECHNICAL TIPS...

Application Instructions

Detailed information is provided in the package insert.

Used as a 10x dilution Aurion ECS serves as a washing medium after immuno incubation and before silver enhancement *(immuno procedure – wash – silver enhancement)*.

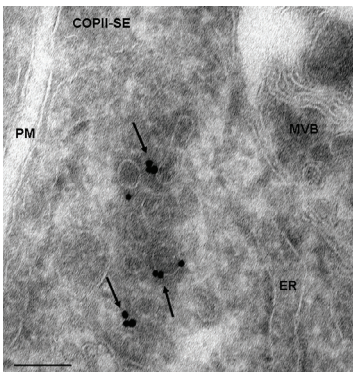
In addition to this application in singe labeling it is required in double labeling experiments as a washing medium between the first silver enhancement step and the second immuno incubation sequence. *(immuno incubation 1 – wash – silver enhancement 1 – wash – immuno incubation 2 – wash – silver enhancement 2)*.

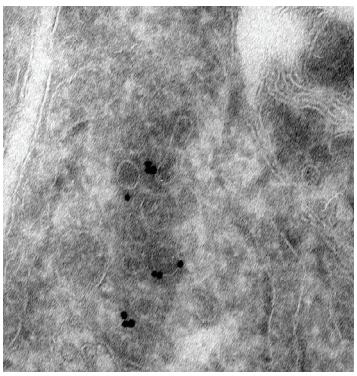
Storage

Aurion ECS has a guaranteed shelf life of 18 months from the date of quality control analysis. Store at 4-8°C. Do not freeze.

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections





ER = endoplasmic reticulum, **PM** = plasma membrane, **MVB** = multi-vesicular body, Bar = 100 micrometer

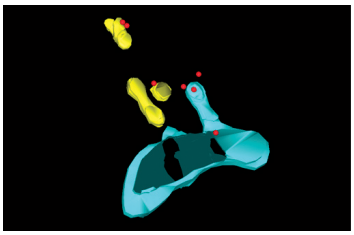
AURION R-Gent SE-EM Application Example

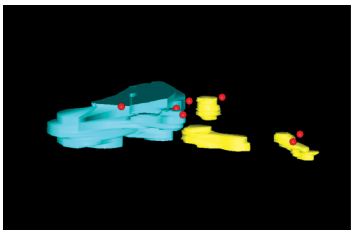
ER exit site in 60 nm-thin cryosection of Hepg2 cells, labeled for COPII (primary antibody against sec23 was obtained by ABR) and detected with Fab-goat-anti-rabbit, conjugated to ultra-small gold, silver enhanced for 30 minutes (from Aurion).

The arrows point to labeled COPII-coats on vesicular and tubular membranes, which are located close to the ER.

The information of a thin section is not sufficient to conclude how the membranes are related to each other- if they are still connected to the ER, or if they are free.

Therefore we performed 3D electron tomography on 400nm thick cryosections, which were labeled similar for COPII. (see next picture).





ER = **light blue**, Free membrane carriers of vesicular and tubular shape, partially labeled for COPII = **yellow**, COPII = **silver enhanced-red**

2 views of a model of a COPII-labeled ER-exit site, resolved from 400nm thick cryo-sections of Hepg2 cells, labeled like described for the ultrathin section before.

Note that the labeling for COPII is assessable throughout the section.

Courtesy of: Dagmar Zeuschner, Judith Klumperman (Department of Cell Biology, UMC Utrecht, The Netherlands) and Willie Geerts, Abraham Koster (Molecular Cell Biology, Utrecht University, The Netherlands)

PRODUCT INFORMATION

AURION

TECHNICAL TIPS...

Application Instructions

Detailed information is provided in the package inserts.

Electron Microscopy

Post-embedding enhancement (on-grid)

Place grids on drops of the enhancement mixture arrayed on a sheet of Parafilm™.

Pre-embedding enhancement

Incubate specimens (e.g. vibratome sections) in Petri dishes or Eppendorf tubes containing the enhancement mixture, with agitation. When enhancement is completed, the specimens are washed with distilled water. A fixation step with photographic fixer is not required.

Light Microscopy

On-slide or -coverslip Labeling

A few drops of the freshly prepared enhancement mixture are applied to cover the specimen. During enhancement the specimens are kept in a moist chamber. The ongoing enhancement may at intervals be monitored with an inverted light microscope. When enhancement is judged to be complete, the specimens are washed with distilled water. A fixation step with photographic fixer is not required.

Whole Mount Labeling

Specimens like floating (vibratome) sections can be enhanced in Petri dishes or 6-24 well plates.

Bio Assays

Depending on the type of assay the enhancement for bio assays can be performed in sealed plastic bags, Petri dishes or in disposable screw cap sealed tubes.

Storage

The AURION R-GENT SE-LM components are stored at 4°C and allowed to reach room temperature before use.

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections

III R-Gent SE-LM Silver Enhancement Reagents

The Immunogold Silver Staining technique (IGSS) finds application both at the electron microscope and the light microscope level. In addition the technique is used to identify proteins or nucleic acids after blotting.

Light microscopical and macroscopical visualization of the latent gold signal requires an enhancement system that renders a high contrast signal. Light insensitivity and negligible autonucleation are required for ease of handling and low background. AURION R-Gent SE-LM is a two-component reagent that meets these requirements.

Introduction

The silver enhancement reaction is based on the gold particle catalyzed reduction of Ag+ to metallic silver using photographic developing compounds as the electron source. For light microscopy and immunoblotting applications the generated silver signal should be of high contrast. Furthermore the signal should be permanent and compatible with counterstaining.

Product Description

The AURION R-GENT SE-LM components constitute a Silver Enhancement Reagent which increases the average gold cluster or particle size by deposition of metallic silver facilitating detection at the light microscopical level. The generated brown-black signal is also easily detected in bio assays and is compatible with counterstaining in light microscopy. AURION R-GENT SE-LM has been tailored for the enhancement of AURION Ultra Small Immunogold reagents and is equally suited for the larger sized particles in the AURION Conventional Immunogold reagents.

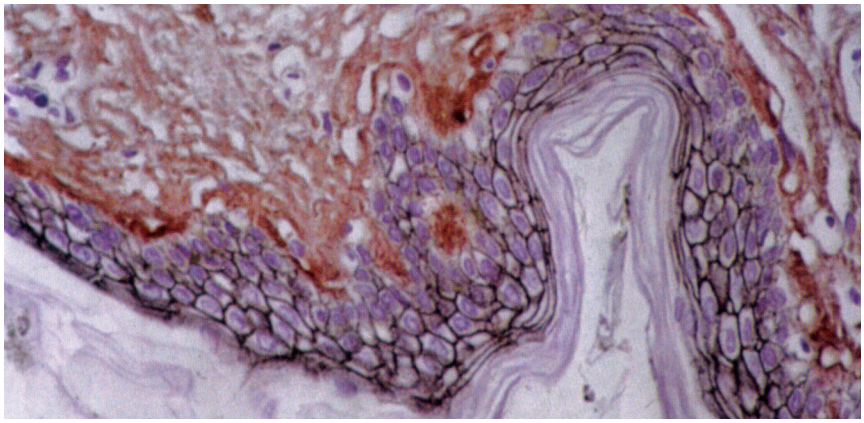
For enhancement, equal amounts of the DEVELOPER and ENHANCER are mixed well and applied to the specimen. The enhancement mixture is easy-to-use, exhibits extremely delayed auto-nucleation and can be used under standard laboratory light conditions. Typical enhancement times are between 15 and 30 minutes. Auto-nucleation becomes visible only after 40-60 minutes. Light microscopical specimens may be counterstained according to standard procedures. The enhancement mixture has a pH value of 8.3-8.5.

AURION R-GENT SE-LM is available as a kit in two unit sizes (2 x 30 ml or 2 x 250 ml) and consists of a separate DEVELOPER and ENHANCER. The supplied amounts accomodate 600 and 5000 LM specimens respectively at 100 µl/specimen, or 60 and 500 bio assay specimens at 1 ml/specimen. The reactivity is tested on dot-spots and the absence of autonucleation is monitored by spectrophotometric techniques.

Light microscopy evaluation of tubulin labeling with Ultra Small Immunogold Reagents and silver enhancement.

Upper panel: bright field mode

Lower panel: epi-polarization mode



Immunogold Silver Staining of E-cadherin on a paraffin section of human skin. Courtesy of R. Moella, Dept. of Exp. Path., EUR, The Netherlands. • Mouse monoclonal anti E-cadherin • GAM IgG UltraSmall • Aurion R-Gent SE-LM

III Gold Nanoparticles – Carboxyl Functionalized

We are proud to announce the introduction of a series of Carboxyl-functionalized Gold Nanoparticles. This product has been developed to facilitate conjugating gold nanoparticles to molecules that cannot be conjugated via the classic direct adsorption method. The product is especially suited for covalent conjugation of small ligands.

Features

- Carboxyl (COOH) functionalized
- Particle sizes 6, 10, 15 and 25nm
- Bind to primary amino amines in target molecule
- Conjugation using EDC/sulfo-NHS chemistry
- Recommended for conjugation of small ligands
- Detailed conjugation protocol
- Ready and easy to use, long term stability

Introduction

Aurion Gold Nanoparticles (Carboxyl Functionalized) are polyethylene glycol-carboxyl stabilized gold nanoparticle solutions based on defined particle sizes of 6, 10, 15 and 25nm.

They are prepared using in-house developed production protocols giving the product a narrow size distribution, long term stability and optimal conjugation properties. Biomolecules having primary amines available can be covalently conjugated using EDC/sulfo-NHS chemistry.

Product Description

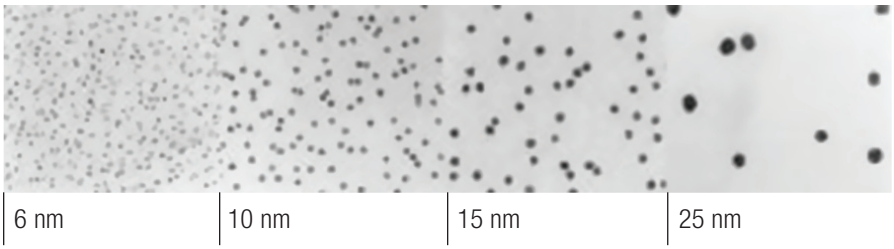
Biomolecules that are too small to be conjugated to Gold Nanoparticles via adsorption can be covalently linked to Carboxyl-functionalized Gold Nanoparticles. Primary amines are present in e.g. the N-terminal side of peptides and in the side group of the amino acid lysine. The conjugation relies on well known and proven EDC/sulfo-NHS chemistry. EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) is a water soluble carbodiimide which transforms the carboxyl groups on the gold to an active ester in the presence of sulfo-NHS (N-Hydroxy-sulfosucciimide, sodium salt). These sulfo-NHS esters are relatively stable in acidic environment and couple rapidly to the amine(s) in the target molecules.

Aurion Gold Nanoparticles (Carboxyl Functionalized) are prepared according to a unique protocol, warranting narrow size distribution, long term stability and optimal conjugation properties. They are available in size ranges 6, 10, 15 and 25 nm. The particle population is monodisperse and thus shows minimal size variation and overlap. Typically, the coefficient of variance for the 6 and 25 nm particle size sols is less than 12%, whereas the 10 and 15 nm size sols show less then 10% variation. Actual lot specifications (size, variation and expiry date) are indicated on the accompanying package insert.

Aurion Gold Nanoparticles (Carboxyl Functionalized) are supplied in 10mM MES buffer, pH 5.0.

Package size: 4x5 ml of high quality Carboxyl-functionalized Gold Nanoparticles at an OD520nm = 1.0.

Particle size and size distributions of the AURION Gold Nanoparticles – Carboxyl Functionalized



NEW SERIES

TECHNICAL TIPS...

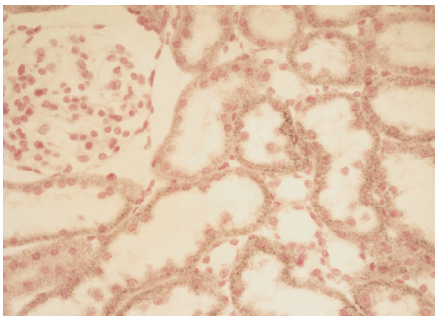
Application Instructions

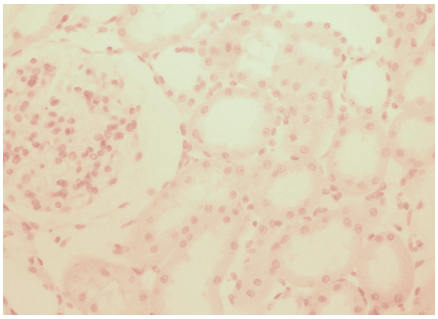
Detailed information is provided in the package inserts.

Storage

Aurion Gold Nanoparticles (Carboxyl Functionalized) have a guaranteed shelf life of 12 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.





Detection of endogenous biotin on formalin fixed rat kidney tissue. After antigen retrieval in 10mM citrate buffer, pH 6, biotin was detected with streptavidin and visualized with 10nm gold conjugated biotin and silver enhancement. Positive signal (top) is mainly present in epithelial cells of proximal tubules. (Bottom) Negative control.

PRODUCT INFORMATION

III Gold Nanoparticles – Gold Sols

To prepare a high quality (immuno)gold conjugate it is important to have particles with uniform size and highly adsorptive surfaces.

The Aurion gold sols are prepared according to unique production protocols. This provides for sol particles with the same narrow size distribution and adsorption properties as employed in the Conventional Immunogold Reagents.

Introduction

The preparation of conventional gold reagents is based on gold particles with a diameter suited for direct electron microscopic visualization. Aurion offers a range of Conventional Immunogold Reagents which cover the majority of approaches in transmission and scanning electron microscopy.

Aurion Gold Sols (solutions of high quality unconjugated gold particles) provide opportunities for users to prepare conjugates with primary antibodies, ligands and other binding agents with the same particle characteristics as in the Conventional Immunogold Reagents.

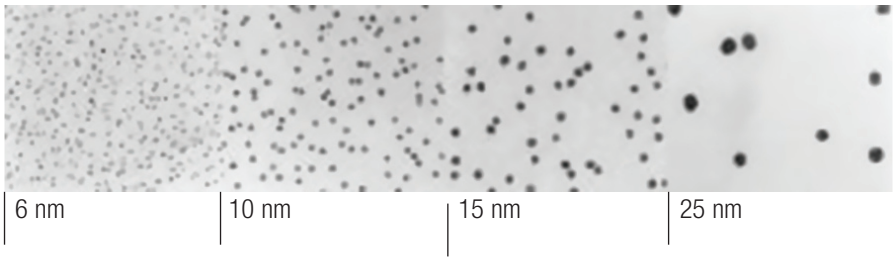
Product Description

AURION Gold Sols are prepared according to a unique protocol, warranting narrow size distribution and reproducible adsorption characteristics.

The AURION gold sols are available in the same size range as the Conventional Immunogold Reagents: 6, 10, 15 and 25 nm. The particle population is monodisperse and thus shows minimal size variation and overlap. Typically, the coefficient of variance for the 6 and 25 nm particle size sols is less than 12%, whereas the 10 and 15 nm size sols show less then 10% variation. Actual lot specifications (size, variation and expiry date) are indicated on the accompanying package insert.

Package size: 100 ml of high quality gold sol at an OD520nm of approximately 1.

Particle size and size distributions of the AURION Gold Nanoparticles – Gold Sols



TECHNICAL TIPS...

Application Instructions

Detailed information is provided in the package inserts.

Storage

AURION gold sols have a guaranteed shelf life of 12 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.

TECHNICAL TIPS...

How to control background in a nutshell

Background Is Controlled by Three Independent Steps

1. Low Molecular Weight Block (before protein block)

Purpose: to inactivate residual fixative e.g. aldehydes, using

- amino acids such as glycin or lysin, or
- aldehyde inactivating compounds such as $NaBH_4$ and NH_2OH

2. High Molecular Weight Protein Block (before immunolabeling)

Purpose: to prevent stickiness to hydrophobic areas and domains with excessive positive charges based on multiple point interactions (high affinity protein binding capacity), using

- albumin
- normal serum

3. Incubation and Wash solution (during immunolabelling)

Purpose: to eliminate aspecific binding of immunoconjugates based on hydrophilic interactions ("oligo" point interactions) by competition, using

- acetylated albumin (AURION BSA-c™) in the incubation buffer

Please refer to our Newsletter 1 and Newsflyer 1 for more information on the subject of background.

You can find incubation protocols in "The Aurion method" section.

III Colloidal Gold Based Protein Stain

Col-Aurion is a colloidal gold particle based total protein stain, developed for the sensitive staining of electrophoretically separated protein bands on nitrocellulose or PVDF™ blotting membranes. Colloidal gold particles accumulate at the site of the protein bands on the membrane, generating an intense dark red staining pattern. The total protein stain assists in assessing immunoblotting results and to evaluate the effectivity of the blotting procedure.

Introduction

The negative surface charge of colloidal gold particles is responsible for their high affinity for positively charged macromolecules. This characteristic was the basis for the development of a total protein stain based on colloidal gold.

Product Description

Col-Aurion is a total protein stain consisting of a solution of coated gold particles with an average particle diameter of 15 nm. The staining principle is based on the electrostatic binding of negatively charged gold particles to proteins with a positive charge present at low pH.

A unique feature of Col-Aurion is the use of BSA-c™ to shield off the surface of the gold particles. Destabilization of gold particles that might occur as a result of interaction with detached protein is thus prevented. The strong negative charge of BSA-c™ gives an additional increase in sensitivity of the stain.

Col-Aurion total protein stain has a pH of 3.2 and is ready to use. For removal of surplus of weakly bound protein from the membrane, a 10 ml vial of Tween-20™ is included.

TECHNICAL TIPS...

Application Instructions

Detailed information is provided in the package insert. Col-Aurion is a sensitive total protein stain for bio assays, especially nitrocellulose and PVDF Western blots and dot-blots. The protein binding substratum has to carry a negative charge.

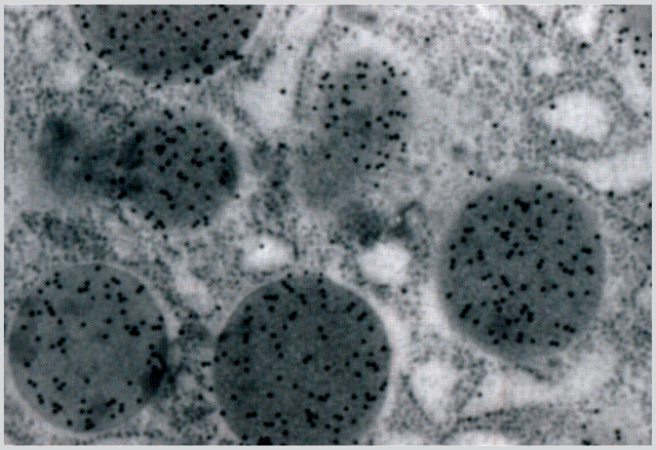
Storage

Col-Aurion has a guaranteed shelf life of 18 months from the date of quality control analysis. Store at 4-8°C. Do not freeze.

Additional Information

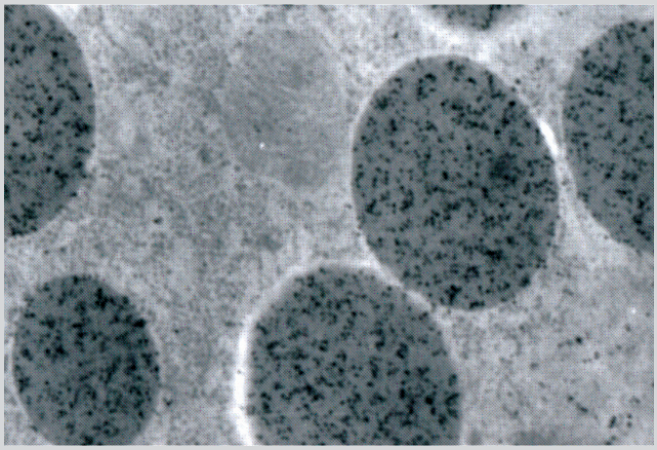
For additional information please see the Aurion Immunogold Newsletter and Protocol sections

MICROGRAPHS



Immunogold silver staining of alpha-amylase on Lowicryl HM20 section of rat pancreas.

- *Goat-anti-Rabbit, 15nm*



Immunogold silver staining of alpha-amylase on Lowicryl HM20 section of rat pancreas.

- *Goat-anti-Rabbit*
- *Ultra Small Silver Enhanced*

PRODUCT INFORMATION

TECHNICAL TIPS...

Application Instructions

In order to obtain a positive reaction when using these tracers the following should be kept in mind:

charge interactions are influenced by the presence of ions, di- and trivalent ions being of more influence than univalent ions. The influence is concentration dependent. Binding is stronger in low ionic-strength media.

charge interactions are influenced by pH. The charge of components in the specimen is dependent of their isoëlectric point, below the IEP the charge is positive, above the IEP negative. Significant binding of the anionic tracer will only occur at a pH at least one to two pH-units lower than the IEP.

Anionic tracers are negatively charged at pH >5

BSA tracers are intended for tracking fine capillaries or connecting spaces in intact tissues.

Storage

Aurion gold tracers have a guaranteed shelf life of 18 months from the date of quality control analysis.

The products should be stored at 4-8°C. Freezing is not recommended.

Additional Information

For additional information please see the Aurion Immunogold Newsletter and Protocol sections.

III Gold Tracers

Aurion Anionic Gold Tracers are used to visualize charged moieties in specimens. They bind to polycationic moieties (basic proteins e.g. histones). "Neutral" BSA coated gold tracers are useful for detecting open connections and tissue damage. Under proper conditions and with suitable microscopical techniques, the tracers can also be used to follow cellular events in time.

Introduction

The AURION Anionic Gold Tracers are designed to detect multiple positive charge moieties.

Product Description

The AURION Gold Tracers are available in the full range of particle sizes: Ultra Small, 6, 10, 15 and 25 nm. Anionic Gold Tracers are prepared with BSA-c™ as particle conjugated protein. AURION Gold Tracers are available in 5 and 10 ml volume packages and are supplied at an OD520nm of 2.0 for the conventional particle size range and at equivalent OD for the Ultra Small tracers.

The products are supplied in PBS, with 15 mM NaN_3.

NOTE 1:
AURION Gold Tracers are also available in bulk and at different optical density if required.

NOTE 2:
AURION Gold Tracers are shipped containing NaN_3 as preservative. If they are intended for use in living organisms, the preservative has to be removed prior to use. This can be achieved either by dialysis or by buffer exchange using, for instance a GE Healthcare Life Sciences PD-10 column.

CUSTOM LABELING

The standard series of AURION ImmunoGold reagents is intended for the two and three step detection of antigens. Although these approaches cover the majority of applications occasionally there may exist a need for a direct label where the gold particles are coupled directly to the primary antibody or, more general, to a protein or peptide with specific binding properties.

To fulfill these needs AURION and EMS offers a custom labeling service for antibodies, antibody fragments, proteins, and peptides with gold particles of any requested size, including ULTRA SMALL gold clusters. AURION designs and manufactures such custom conjugates in close cooperation and in agreement with the principal investigator.

Starting material

As a rule AURION will only accept pure substances as starting material, either lyophilized or dissolved in a medium of known chemical composition. Interfering substances must not be present.

Quality control criteria

The custom prepared product is quality controlled by AURION according to our in-house standards with respect to size distribution and clustering. In agreement with the principal investigator the binding substance's specific quality control criteria are determined. These are tailored for each individual assignment. As a matter of course the custom conjugate is approved only when both sets of quality control criteria have been met.

A Few Examples

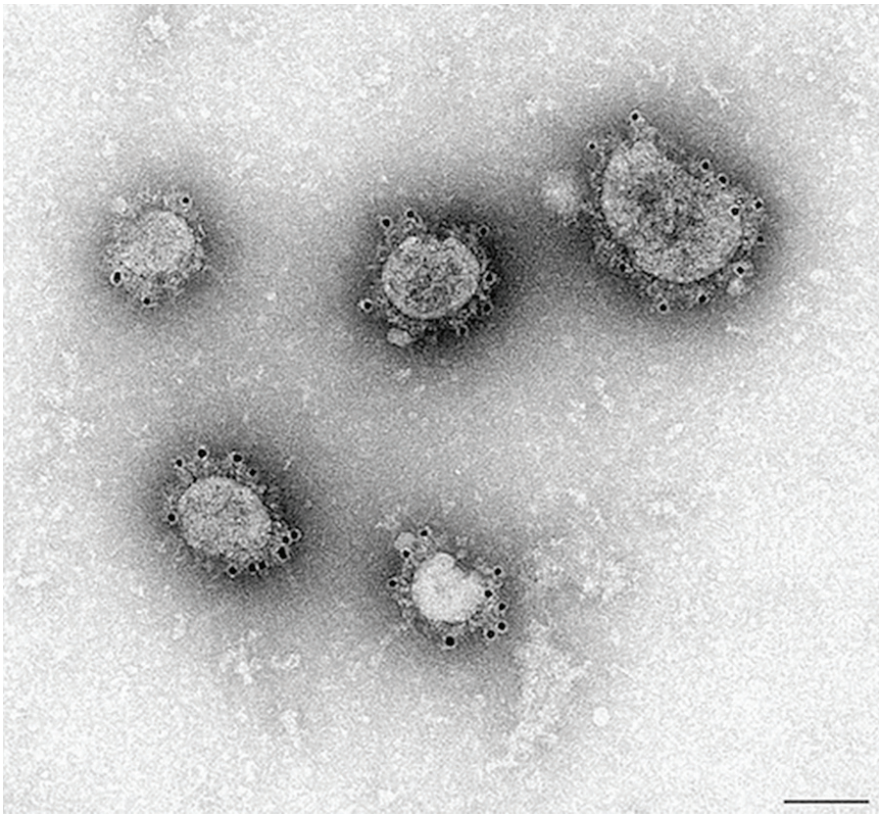
- **AURION successfully conjugated a number of polyclonal antibodies, mouse monoclonal antibodies, F(ab')$_2$ and F(ab) fragments, high and low density lipoproteins, as well as peptides such as an Angiotensin derivative (octapeptide), Interleukin II, Tumor Necrosis Factor and a number of peptide hormones.**
- **The efficiency of coupling (recovery of protein) for the above mentioned custom conjugates has been greater than 70% on all occasions, while efficiencies up to 90% are no exception.**

No cure, no pay

The custom labeling service is on the basis of "no cure, no pay," with the exception that an intake fee applies to each actual laboratory attempt.

We invite you to contact us directly if you require information which is more tailored to your specific applications.

APPLICATION EXAMPLES

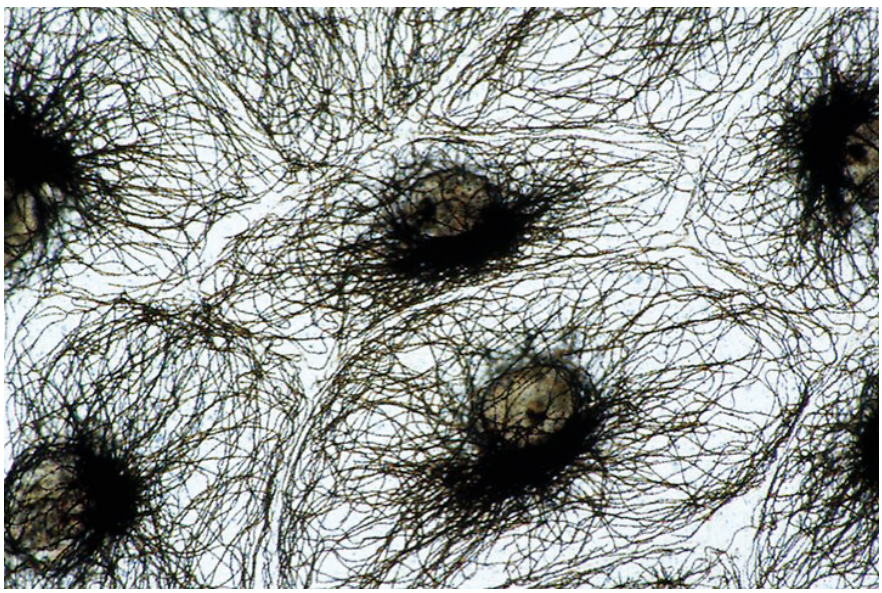


AURION Conventional Reagents Application Example

Immunogold labeling of Middle East Respiratory Syndrome-coronavirus (MERS-CoV).

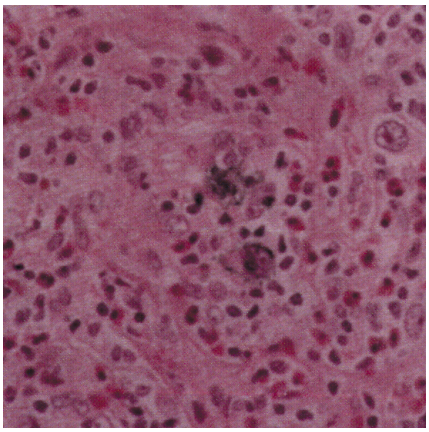
MERS virus is grown in VERO cells via a grid cell culture technique. Primary antibody is a camel anti MERS serum which reacts with the glycoprotein spikes of the virus. Detection with Protein A 10nm. After incubation with the gold reagent specimens are fixed in glutaraldehyde and negative stained with Nano-W.

Courtesy of Sandra Crameri, Australian Animal Health laboratory CSIRO, Geelong, Australia

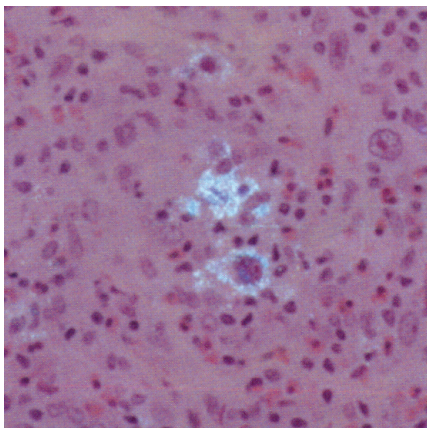


IGSS of tubulin on coverslip culture of PtK2 cells Courtesy of Peter van de Plas, Aurion, Wageningen, The Netherlands.

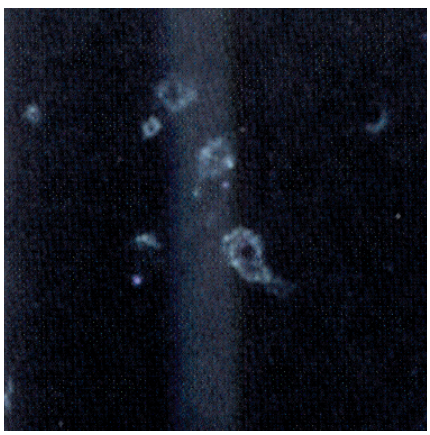
Routine paraffin section of Hodgkin lymphoma stained for CD 15. Reed-Sternberg cells show positive staining in the cytoplasm.
PRODUCTS USED: • Mouse monoclonal CD 15 • GAM IgG UltraSmall • Aurion R-Gent SE-LM
Courtesy of H. Stoop, Laboratory of Pathology and Anatomy, Dordrecht, The Netherlands.



Regular Light Microscopy



Combination regular light microscopy and epi-polarization microscopy



Epi-polarization microscopy

ORDERING INFORMATION

Goat-anti-Rabbit IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25100**	1.5	**25101**	.6
4-8°C	EM-grade 2nm	**25105**	1.0	**25106**	.4
4-8°C	EM-grade 6nm	**25103**	2.5	**25104**	1.0
4-8°C	EM-grade 10nm	**25108**	2.5	**25109**	1.0
4-8°C	EM-grade 15nm	**25112**	3.5	**25113**	1.4
4-8°C	EM-grade 25nm	**25115**	3.5	**25116**	1.4

Goat-anti-Mouse IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25120**	1.5	**25121**	.6
4-8°C	EM-grade 2nm	**25125**	1.0	**25126**	.4
4-8°C	EM-grade 6nm	**25123**	2.5	**25124**	1.0
4-8°C	EM-grade 10nm	**25128**	2.5	**25129**	1.0
4-8°C	EM-grade 15nm	**25132**	3.5	**25133**	1.4
4-8°C	EM-grade 25nm	**25135**	3.5	**25136**	1.4

Goat-anti-Mouse IgM (µ-chain)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25140**	1.5	**25141**	.6
4-8°C	EM-grade 6nm	**25143**	2.5	**25144**	1.0
4-8°C	EM-grade 10nm	**25148**	2.5	**25149**	1.0
4-8°C	EM-grade 15nm	**25152**	3.5	**25153**	1.4
4-8°C	EM-grade 25nm	**25155**	3.5	**25156**	1.4

Goat-anti-Mouse IgG+IgM

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25160**	1.5	**25161**	.6
4-8°C	EM-grade 6nm	**25163**	2.5	**25164**	1.0
4-8°C	EM-grade 10nm	**25168**	2.5	**25169**	1.0
4-8°C	EM-grade 15nm	**25172**	3.5	**25173**	1.4
4-8°C	EM-grade 25nm	**25175**	3.5	**25176**	1.4

Goat-anti-Rat IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25180**	1.5	**25181**	.6
4-8°C	EM-grade 6nm	**25183**	2.5	**25184**	1.0
4-8°C	EM-grade 10nm	**25188**	2.5	**25189**	1.0
4-8°C	EM-grade 15nm	**25192**	3.5	**25193**	1.4
4-8°C	EM-grade 25nm	**25195**	3.5	**25196**	1.4

Goat-anti-Human IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25200**	1.5	**25201**	.6
4-8°C	EM-grade 6nm	**25203**	2.5	**25204**	1.0
4-8°C	EM-grade 10nm	**25208**	2.5	**25209**	1.0
4-8°C	EM-grade 15nm	**25212**	3.5	**25213**	1.4
4-8°C	EM-grade 25nm	**25215**	3.5	**25216**	1.4

Rabbit-anti-Goat IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25220**	1.5	**25221**	.6
4-8°C	EM-grade 6nm	**25223**	2.5	**25224**	1.0
4-8°C	EM-grade 10nm	**25228**	2.5	**25229**	1.0
4-8°C	EM-grade 15nm	**25232**	3.5	**25233**	1.4
4-8°C	EM-grade 25nm	**25235**	3.5	**25236**	1.4

Goat-anti-Biotin

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25240**	1.5	**25241**	.6
4-8°C	EM-grade 6nm	**25243**	2.5	**25244**	1.0
4-8°C	EM-grade 10nm	**25248**	2.5	**25249**	1.0
4-8°C	EM-grade 15nm	**25252**	3.5	**25253**	1.4
4-8°C	EM-grade 25nm	**25255**	3.5	**25256**	1.4

Streptavidin

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25260**	1.5	**25261**	.6
4-8°C	EM-grade 6nm	**25263**	2.5	**25264**	1.0
4-8°C	EM-grade 10nm	**25268**	2.5	**25269**	1.0
4-8°C	EM-grade 15nm	**25272**	3.5	**25273**	1.4
4-8°C	EM-grade 25nm	**25275**	3.5	**25276**	1.4

Protein A

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	EM-grade 6nm	**25282**	2.5	**25283**	1.0
4-8°C	EM-grade 10nm	**25284**	2.5	**25285**	1.0
4-8°C	EM-grade 15nm	**25286**	3.5	**25287**	1.4
4-8°C	EM-grade 25nm	**25288**	3.5	**25289**	1.4

Biotinylated Albumin

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25290**	1.5	**25291**	.6
4-8°C	EM-grade 6nm	**25293**	2.5	**25294**	1.0
4-8°C	EM-grade 10nm	**25298**	2.5	**25299**	1.0
4-8°C	EM-grade 15nm	**25302**	3.5	**25303**	1.4
4-8°C	EM-grade 25nm	**25305**	3.5	**25306**	1.4

Mouse Monoclonal anti-FITC

Gold Conjugates:

For the electron microscopic visualization of FITC labels used in light microscopic specimens. For retrieval of faded FITC in DNA/RNA probes, fatty acids, and proteins.

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25580**	1.5	**25581**	.6
4-8°C	EM-grade 10nm	**25582**	2.5	**25583**	1.0

Goat-anti-Chicken IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25584**	1.5	**25585**	.6
4-8°C	EM-grade 6nm	**25586**	2.5	**25587**	1.0
4-8°C	EM-grade 10nm	**25588**	2.5	**25589**	1.0
4-8°C	EM-grade 15nm	**25590**	3.5	**25591**	1.4
4-8°C	EM-grade 25nm	**25592**	3.5	**25593**	1.4

Protein G

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	EM-grade 6nm	**25312**	2.5	**25313**	1.0
4-8°C	EM-grade 10nm	**25314**	2.5	**25315**	1.0
4-8°C	EM-grade 15nm	**25316**	3.5	**25317**	1.4
4-8°C	EM-grade 25nm	**25318**	3.5	**25319**	1.4

Goat-anti-Guinea Pig IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25320**	1.5	**25321**	.6
4-8°C	EM-grade 6nm	**25323**	2.5	**25324**	1.0
4-8°C	EM-grade 10nm	**25328**	2.5	**25329**	1.0
4-8°C	EM-grade 15nm	**25332**	3.5	**25333**	1.4
4-8°C	EM-grade 25nm	**25335**	3.5	**25336**	1.4

Rabbit-anti-Sheep IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4-8°C	Ultra-Small	**25340**	1.5	**25341**	.6
4-8°C	EM-grade 6nm	**25343**	2.5	**25344**	1.0
4-8°C	EM-grade 10nm	**25348**	2.5	**25349**	1.0
4-8°C	EM-grade 15nm	**25352**	3.5	**25353**	1.4
4-8°C	EM-grade 25nm	**25355**	3.5	**25356**	1.4

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ORDERING INFORMATION

TECHNICAL TIPS...

Reactivity of Protein A and Protein G

Membrane labeling with protein A 15 nm gold conjugate. Courtesy of T.Wakefield, University of Auburn, Alabama

Species	Ig(sub)class	Protein A	Protein G
Rabbit	Ig	++	++
Mouse	IgG1	-	+
	IgG2a	+	+
	IgG2b	+	+
	IgG3	+	++
	IgA	-	?
	IgM	-	?
Rat	IgG1	-	?
	IgG2a	-	++
	IgG2b	-	+
	IgG2c	+	+
	IgM	-	?
Man	IgG1	++	++
	IgG2	++	++
	IgG3	-	++
	IgG4	++	++
	IgA	-	-
	IgM	+	-
	IgD	-	-
Goat	Ig	-	++
Sheep	Ig	-	++
Guinea Pig	Ig	++	+
Chicken	Ig	-	-
Pig	Ig	++	+
Horse	Ig	-	++
Cow	Ig	+	++
Dog	Ig	+	+

F(ab')$_2$ Fragment of Goat-anti-Rabbit IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25360**	1.5	**25361**	.6
4–8°C	EM-grade 6nm	**25363**	2.5	**25364**	1.0
4–8°C	EM-grade 10nm	**25362**	2.5	**25365**	1.0
4–8°C	EM-grade 15nm	**25366**	3.5	**25367**	1.4
4–8°C	EM-grade 25nm	**25368**	3.5	**25369**	1.4

F(ab')$_2$ Fragment of Goat-anti-Mouse IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25370**	1.5	**25371**	.6
4–8°C	EM-grade 6nm	**25373**	2.5	**25374**	1.0
4–8°C	EM-grade 10nm	**25372**	2.5	**25375**	1.0
4–8°C	EM-grade 15nm	**25376**	3.5	**25377**	1.4
4–8°C	EM-grade 25nm	**25378**	3.5	**25379**	1.4

F(ab')$_2$ Fragment of Goat-anti-Mouse IgG+IgM

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25380**	1.5	**25381**	.6
4–8°C	EM-grade 6nm	**25383**	2.5	**25384**	1.0
4–8°C	EM-grade 10nm	**25382**	2.5	**25385**	1.0
4–8°C	EM-grade 15nm	**25386**	3.5	**25387**	1.4
4–8°C	EM-grade 25nm	**25388**	3.5	**25389**	1.4

F(ab) Fragment of Sheep-anti-Digoxigen

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25410**	1.5	**25411**	.6
4–8°C	EM-grade 10nm	**25398**	2.5	**25399**	1.0

F(ab') Fragment of Goat-anti-Rabbit IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25412**	1.5	**25413**	.6

F(ab') Fragment of Goat-anti-Mouse IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25414**	1.5	**25415**	.6

F(ab') Fragment of Goat-anti-Rat IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25416**	1.5	**25417**	.6

Rabbit-anti-Chicken IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25420**	1.5	**25421**	.6
4–8°C	EM-grade 6nm	**25423**	2.5	**25424**	1.0
4–8°C	EM-grade 10nm	**25428**	2.5	**25429**	1.0
4–8°C	EM-grade 15nm	**25432**	3.5	**25433**	1.4
4–8°C	EM-grade 25nm	**25435**	3.5	**25436**	1.4

III Cross Adsorbed Products

Cross reactivity can be a problem when working with primary antibodies sourced from animal species that are evolutionary closely related to the source of tissues or cells that antigens are to be detected in.

An example is using a mouse monoclonal on rat tissue. With many monoclonal antibodies of mouse origin and some of rat, and the prevailing animal cells and tissues being used in studies likewise originating from rat or mouse, it often happens that cross reactivity occurs, leading to false positive results.

A second problem that derives from cross-reactivity occurs in double labeling when using primary antibodies from closely related animal sources. With this in mind, Aurion has developed two series of new conjugates:

Goat-anti-Mouse IgG (H+L)xRat (cross adsorbed against Rat immunoglobulins)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	EM-grade Ultra Small	**25900**	1.5	**25901**	0.6
4–8°C	EM-grade 10nm	**25902**	2.5	**25903**	1.0

Goat-anti-Rat IgG (H+L)xMouse (cross adsorbed against Mouse immunoglobulins)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	EM-grade Ultra Small	**25904**	1.5	**25905**	0.6
4–8°C	EM-grade 10nm	**25906**	2.5	**25907**	1.0

Secondary antibodies have been thoroughly purified using solid phase technique. These purified antibodies allow detection of antigens with mouse monoclonal antibodies in rat tissue or rat monoclonal antibodies in mouse tissue, without causing interfering binding to either endogenous mouse or rat immunoglobulins.

The antibodies can be used for double labeling as well when both mouse and rat primary antibodies are used with other than mouse or rat tissue.

Please note:

Cross adsorption against closely related species may result in epitope recognition with reduced binding force and lower labeling density.

EMS and Aurion would like to inform you that the strongest labeling intensity is usually achieved with the least refined antibodies. Users are advised to take this into account when choosing a product from the Aurion range of anti-mouse or anti-rat conjugates. In case of doubt, our technical staff will gladly assist you in making the best choice.

ORDERING INFORMATION

Donkey Conjugate Line

In many cases double labeling experiments are based on using two primary antibodies from different animal species, for instance mouse and rabbit. Of late, we have noticed an increasing number of users that have primary antibodies developed in goat. It is impossible to perform a double labeling experiment in combination with, for instance a mouse primary antibody, since applicable secondary antibodies are either raised in rabbit or goat. Hence the Goat-anti-Mouse would be recognized by the Rabbit-anti-Goat, and it would be impossible to get reliable results. In the past, only using directly labeled primary antibodies would solve this. This would require researchers to go through the lengthy procedure of preparing such conjugates and even then with limited success.

To overcome this difficulty we are proud to release the Aurion Series of Secondary ImmunoGold conjugates based on antibodies raised in Donkey, including Donkey-anti-Goat. It is then known that mixing Donkey-anti-Goat and Donkey-anti-Mouse provides a successful solution to the above mentioned case.

Donkey-anti-Rabbit IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-small	**25700**	1.5	**25701**	0.6
4–8°C	EM-grade 6nm	**25702**	2.5	**25703**	1.0
4–8°C	EM-grade 10nm	**25704**	2.5	**25705**	1.0
4–8°C	EM-grade 15nm	**25706**	3.5	**25707**	1.4
4–8°C	EM-grade 25nm	**25708**	3.5	**25709**	1.4

Donkey-anti-Goat IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25800**	1.5	**25801**	0.6
4–8°C	EM-grade 6nm	**25802**	2.5	**25803**	1.0
4–8°C	EM-grade 10nm	**25804**	2.5	**25805**	1.0
4–8°C	EM-grade 15nm	**25805**	3.5	**25807**	1.4
4–8°C	EM-grade 25nm	**25808**	3.5	**25809**	1.4

Donkey-anti-Mouse IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25810**	1.5	**25811**	0.6
4–8°C	EM-grade 6nm	**25812**	2.5	**25813**	1.0
4–8°C	EM-grade 10nm	**25814**	2.5	**25815**	1.0
4–8°C	EM-grade 15nm	**25816**	3.5	**25817**	1.4
4–8°C	EM-grade 25nm	**25818**	3.5	**25819**	1.4

Donkey-anti-Sheep IgG (H&L)

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25820**	1.5	**25821**	0.6
4–8°C	EM-grade 6nm	**25822**	2.5	**25823**	1.0
4–8°C	EM-grade 10nm	**25824**	2.5	**25825**	1.0
4–8°C	EM-grade 15nm	**25826**	3.5	**25827**	1.4
4–8°C	EM-grade 25nm	**25828**	3.5	**25829**	1.4

BSA-Fragment Stabilized

Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
Ultra-Small Gold	**25498**	20	—	—

Gold Nanoparticles – Carboxyl Functionalized

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	6nm	**25600**	4x5	—	—
4–8°C	10nm	**25601**	4x5	—	—
4–8°C	15nm	**25602**	4x5	—	—
4–8°C	25nm	**25603**	4x5	—	—

Gold Nanoparticles – Gold Sols

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25509**	20	—	—
4–8°C	6nm	**25510**	100	—	—
4–8°C	10nm	**25512**	100	—	—
4–8°C	15nm	**25514**	100	—	—
4–8°C	25nm	**25516**	100	—	—

Gold Tracers

Anionic Gold Tracer

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25460**	10	**25461**	5
4–8°C	EM-grade 6nm	**25463**	10	**25464**	5
4–8°C	EM-grade 10nm	**25466**	10	**25467**	5
4–8°C	EM-grade 15nm	**25469**	10	**25470**	5
4–8°C	EM-grade 25nm	**25472**	10	**25473**	5

BSA Gold Tracer

	Particle Size	Cat #	Size(ml)	Cat #	Size(ml)
4–8°C	Ultra-Small	**25480**	10	**25481**	5
4–8°C	EM-grade 6nm	**25483**	10	**25484**	5
4–8°C	EM-grade 10nm	**25486**	10	**25487**	5
4–8°C	EM-grade 15nm	**25489**	10	**25490**	5
4–8°C	EM-grade 25nm	**25492**	10	**25493**	5

Blocking Solutions

	Particle Size	Cat #	Size(ml)
4–8°C	Basic Blocking (For Protein A&G)	**25595**	30
4–8°C	Goat Gold Conjugates	**25596**	30
4–8°C	Rabbit Gold Conjugates	**25597**	30
4–8°C	Sheep Gold Conjugates	**25598**	30
4–8°C	Donkey Gold Conjugates	**25599**	30

R-Gent Silver Enhancement, Blocking Solutions, Background Suppression

	Cat. #	Description	Size(ml)
4–8°C	**25520**	AURION R-Gent SE-LM Kit	2x30ml
4–8°C	**25522**	AURION R-Gent SE-LM Kit	2x250ml
4–8°C	**25521**	AURION R-Gent SE-EM Kit	30ml
4–8°C	**25521-90**	AURION R-Gent SE-EM Kit	90ml
4–8°C	**25536**	COL-AURION Total Protein Stain	500ml
4–8°C	**25554**	Bovine Serum Albumin	25gr
4–8°C	**25558**	AURION BSA-c™ (acetylated BSA)	100ml
4–8°C	**25557**	AURION BSA-c™ (acetylated BSA)	30ml
4–8°C	**25560**	Cold Water Fish Skin Gelatin 40%	10ml
4–8°C	**25564**	Tween-20	10ml
4–8°C	**25568**	Normal Rabbit Serum	5ml
4–8°C	**25570**	Normal Goat Serum	5ml
4–8°C	**25574**	Gum Arabic	20gr
4–8°C	**25578**	Normal Sheep Serum	5ml
4–8°C	**25572**	Normal Donkey Serum	5ml
4–8°C	**25830**	Enhancement Conditioning Solution	100ml

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III Immunogold Reagent Kits – EM Grade

Complete kits with the following characteristics:

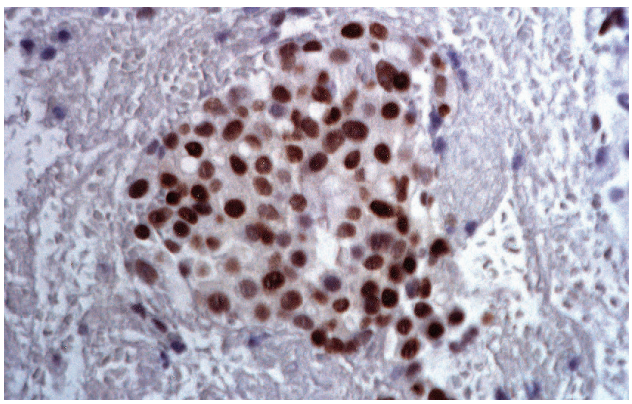
- Two non-overlapping gold particle sizes.
- Coupled to: either Goat-anti-Rabbit and Goat-anti-Mouse IgG/IgM antibodies.
- Affinity purified and cross adsorbed antibodies.
- Unparalleled low background levels.
- For use with rabbit and mouse monoclonal and polyclonal primary antibodies.
- Acetylated serum albumin with increased exposure of negative charges.

Benefits:

- Covering over 90% of all applications in EM.
- For single and double labeling in Electron Microscopy

Cat. #	Description
4–8°C **25540**	EM-kit GAR-6/GAM-10/BSA-c™ ***Kit consists of:*** • 1ml Goat-anti-Rabbit 6nm • 1ml Goat-anti-Mouse IgG 10nm • 30ml BSA-c™ background suppressing agent
4–8°C **25544**	EM-kit GAM-6/GAR-10/BSA-c™ ***Kit consists of:*** • 1ml Goat-anti-Mouse IgG 6nm • 1ml Goat-anti-Rabbit 10nm • 30ml BSA-c™ background suppressing agent
4–8°C **25548**	EM-kit PrA-6/PrA-10/BSA-c™ ***Kit consists of:*** • 1ml Protein A 6nm • 1ml Protein A 10nm • 30ml BSA-c™ background suppressing agent
4–8°C **25540-05**	Custom EM Kit

For customization of our EM Kits you get to choose the 2 vials of your EM Grade Reagent. Supplied with the kit as well is 30ml of BSA-c™.



Immuno-peroxidase staining of estrogen receptor on paraffin section of bronchus carcinoma. Incubation using Aurion BSA-c™

Courtesy of Hans Stoop, Lab of Pathologpy and Anatomy, Dordrecht, The Netherlands

Benefits of our US-KIT:

For EM:

- For the most sensitive labeling in electron microscopy
- For the penetration in ultra thin cryosections resulting in enhanced detectability and sensitivity
- New possibilities for pre-embedding labeling
- For the labeling of at least 330 on-grid specimens

For LM:

- Premium reagents for penetration in hydrated sections (paraffin, cryostat) and in slightly fixed cells
- Permanent, non-fading silver signal
- Most sensitive reagents compatible with counterstaining
- For the immunolabeling of 200 slides

For Immunoblotting:

- Highest sensitivity
- Black to brown/black signal suited for low level detection where colored precipitates fail
- For the immunogold labeling of 10-20 nitrocellulose immunoblot strips

Cat. #	Description
4–8°C **25550**	US-kit GAR/GAM/BSA-c™/R-Gent SE-LM ***Kit consists of:*** • 0.6ml Goat-anti-Rabbit GP-Ultra-Small • 0.6ml Goat-anti-Mouse IgG GP-Ultra-Small • 30ml BSA-c™ background suppressing agent • SE-LM Silver Enhancement
4–8°C **25552**	US-kit GAR/GAM/BSA-c™/R-Gent SE-EM ***Kit consists of:*** • 0.6ml Goat-anti-Rabbit GP-Ultra-Small • 0.6ml Goat-anti-Mouse IgG GP-Ultra-Small • 30ml BSA-c™ background suppressing agent • SE-EM Silver Enhancement
4–8°C **25550-10**	Ultra-Small Starter Kit

To give yourself the opportunity to make yourself familiar with our Ultra-Small Gold Conjugates – we have our Starter Kit. These kits are sufficient for approximately 50 specimens.

Kit consists of:

- 100 µl Ultra-Small Conjugate
- 10ml BSA-c™
- 2x2.5ml R-Gent SE-LM and a sample pack R-Gent SE-EM

4–8°C **25550-05** Custom Ultra-Small Kit with SE-LM

For customization of our Ultra-Small Kits we now have available a make it yourself kit where you get to choose the two vials of your Ultra-Small Reagent. Also supplied with the kit is 30ml BSA-c™ and 2x30ml SE-LM

4–8°C **25550-06** Custom Ultra-Small Kit with SE-EM

For customization of our Ultra-Small Kits we now have available a make it yourself kit where you get to choose the two vials of your Ultra-Small Reagent. Also supplied with the kit is 30ml BSA-c™ and 30ml SE-EM.

FREQUENTLY ASKED QUESTIONS

We have collected answers to frequently asked questions from immunogold users. They are listed below. If your question is not listed on this page or you have further queries, please contact us directly by phone or e-mail. We remain at your disposal.

What kind of particle size should I use?

Always use the smallest particle size to fit your application. Conjugates based on smaller particles are more efficient than larger particle based conjugates. If visualization is difficult with smaller particles these can be enlarged with silver enhancement. Very sensitive specimens for SEM observation are best served with a larger particle size conjugate. This prevents ultra structural enhancement reagents.

Is it true that gold conjugates are more background prone than other conjugates?

No! This fairy tale comes from the fact that gold conjugates are based on particles and that visualization is also based on separate particles. Contrary to enzyme and fluorescent markers, gold conjugates are more like a digital system, either they are there and then you will see them, or they are not present. Enzyme and fluorescent markers are sooner to be considered as "analogue" markers, their visibility in detection increases with their local concentration or with the time the enzyme marker can produce a visible reaction product. An unbiased look at controls in fluorescence shows always a low level of light that is inherent to the presence of double bonds in biological compounds and on top of this comes the fluorescence from the labeled antibodies. Likewise will an unbiased look at control specimens incubated only with alkaline phosphatase or peroxidase labeled antibodies usually show a faint overall staining of the specimen. Such faint levels are easily accepted or even filtered out. You cannot do this with gold conjugates since they are based on particles.

Should I use a secondary gold conjugate or Protein A (or G)?

That depends on what your goal is. Using secondary conjugates results in a higher labeling density. Therefore it is often said that secondary conjugates are more sensitive than Protein A conjugates. This is partly true. Protein A (or G) recognizes only one site on a primary antibody molecule. Binding will occur only when this site is available and not obscured by its environment. Secondary conjugates recognize more sites on primaries and therefore the chance that a primary antibody will be detected is greater. Essentially this is the increase in sensitivity.

If all primary antibodies would be available to the sane extent for binding to either Protein A or a secondary antibody conjugate, then the use of the latter would only result in more particles. This helps in localizing antigens at low magnifications, in other words this is an increase in detectability.

Is there a training program for immunogold (silver) staining where I can bring my own specimens?

EMS and Aurion organizes wet-workshops in Europe and the USA where you preferably work with your own specimens and primary antibodies. After all, that is where your interest lies. If required, we will expand our activities to additional venues. The workshops last for two days and give an in-depth view in immunogold (silver) staining. Detailed information on the setup of our workshops can be found in this publication.

Is it possible to do pre-embedding labeling of intracellular antigens?

Yes. Single cells are most suited. Plant material with a thick impenetrable wall is not. The Ultra-Small gold conjugates are the conjugates of choice. In many cases a permeabilization step with $NaBH_4$ suffices to open up the specimens and allow penetration of reagents. Low concentrations of mild detergents like saponin help. One thing should be emphasized: reaction times have to be prolonged since full penetration of the reagents to the internal antigens has to be achieved. To remove unreacted reagents after incubation wash procedures have to be adapted likewise! The Aurion Newsletter #5 deals with this topic.

How can I verify that my conjugates are still active?

There is a simple procedure to check this. It is described in great detail in Aurion's Newsletter #4. In short: you need a nitro-cellulose strip, apply dots from a dilution series of your primary antibody and incubate the strip with the gold reagent. The dots will stain red with the larger conjugates. When testing an Ultra-Small conjugate silver enhancement has to be applied for visualization.

How can I verify that the silver enhancement reagents are still fine?

Again, there is a simple procedure to check this. It is described in great detail in our Newsletter #4. In short: you need a nitro-cellulose strip, apply dots from a dilution series of your gold conjugate and incubate the strip with the silver enhancement reagents. The dots should become brown-black. During this period of time the mix of reagents should remain glass clear without any visible presence of silver caused by auto nucleation.

The activity of the Silver Enhancement reagent SE-EM for Electron microscopy can be tested by adding 10µl of the enhancement mix. The solution s should turn yellow in 30-45 minutes.

Is it advisable to use outdated conjugates?

As long as their reactivity is OK and there are not too many clusters formed this is no problem. Gold conjugates are very stable. There may be some release of protein from the particle surface with time, but generally this does not result in reduced reactivity. The reactivity of the conjugate is easily checked with a dot-spot test as described in Newsletter #4. Cluster formation may increase with time, depending on the type of conjugated protein and the particle size. The larger the particles the more clusters. These can be removed by centrifugation of the diluted conjugate before use.

Is it possible to double label using two antibodies from the same animal source?

Yes, there are ways to do this. One is by using Protein G or Protein A conjugates with different particle sizes. The procedure would be: first incubate with primary antibody I, detect this with Protein A (or G) with the smaller particle size. Then incorporate an incubation with excess free Protein A or G (50-100 µg/ml). This will block practically all binding sites for Protein A or G. Next, incubate for the second antigen with primary antibody II and detect this with the larger sized Protein A or G gold conjugate.

A second possibility is to use one-step incubations with a mix of primary antibodies, each labeled directly with a different gold particle size. We offer a custom labeling service. Details can be found in the section on custom labeling.

What kind of grids should I use for silver enhancement?

Nickel is the material of choice. Gold grids are out of the question as they will be neatly enhanced as well. The same with copper. Nickel grids are preferred to copper ones for immuno incubations anyway, since nickel is more inert and less poisonous to immuno or enzyme reactions.

Nickel grids can be annoying because of their magnetic properties. This is easily overcome by using either non-magnetic tweezers or by using a flattened loop to transfer grids from droplet to droplet during immuno incubations. We do sell a perfect-loop (70944) for this application.

What about silver enhancement and OsO_4?

OSO_4 fixation can be used before incubation, after incubation or after silver enhancement.

- Because of its destructive effect on antigens OSO_4 fixation is not often used when immuno incubations are intended. However, in general silver enhancing immuno incubated OSO_4 fixed specimens causes no difficulties.
- An Osmium fixation step can be introduced after incubation to improve contrast in specimens. As stated before, applying silver enhancement generally causes no difficulties.
- On occasion in the past, using OSO_4 fixation after silver enhancement used to lead to the removal of part of the deposited silver. However, with the introduction of SE-EM^{plus} this is no longer the case, as the resulting enhanced particles are no longer sensitive to oxidation.

I get no positive results, now what?

When your incubated specimens look as clean as your controls, either (one or more of) the reagents are inactive, or the antigens are destroyed, masked or absent. The cause is easily found by performing tests working backwards through the incubation protocol using dot-spot tests as described in Newsletter #4.

First test the activity of the silver enhancement reagents (if they were used at all) on the gold conjugate that was used. If silver enhancement is fine, the next step is to test the gold conjugate on the primary antibody used and so on.

If it proves that the problem is not in the reagents, you will have to look into antigen preservation. Is a different fixation due? Or a different embedding medium? Using light microscopical evaluation of the results such questions are answered without tedious EM experimental work.

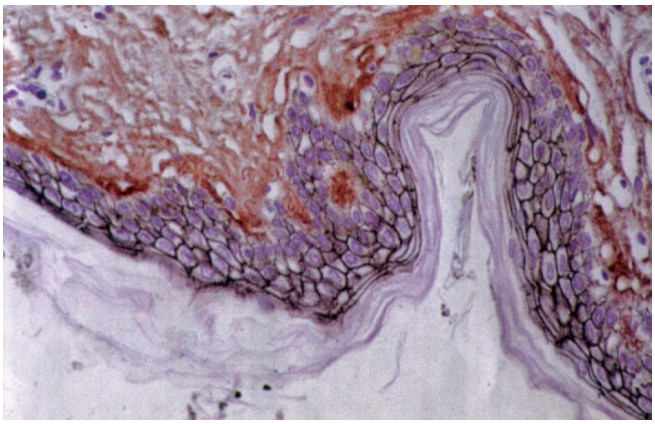
I am having background problems. Is this due to the gold conjugate?

When specimens are blocked correctly and the right composition and condition of incubation buffer is used, background levels should not be interfering with specific signals. Some background will always exist: to some extent all compounds have a certain affinity for other compounds and depending on availability and concentration an interaction may occur. There is no absolute black and white in this respect.

When you leave out the primary antibody incubation and only use the gold step and your background has become much reduced, then your primary antibody causes background. Remedy: purify the primary antibody by either affinity chromatography (in case of an antiserum) and/or by cross-adsorption. If you have unacceptable levels of background without using a primary incubation, then the specimen has a tendency to bind to gold conjugates.

Background may have many causes which are centered around three different types of interactions:

- Residual fixative activity, which is eliminated by using a $NaBH_4$ or Glycine block step prior to the protein block step.
- Stickiness to hydrophobic areas (embedding medium, lipid rich specimen compounds). This is reduced by using an adequate protein block step involving a partly hydrophobic protein like BSA or Casein.
- Charge-based interactions causing negatively charged reagents such as antibodies and gold conjugates to adhere to oppositely charged areas in the specimen (notorious are the histone proteins, some collagen types and poly-L-lysine that is sometimes used to make sections stick to surfaces). This type of interaction can only be overcome by adding an excess of negatively charged indifferent molecules to the incubation media. We have developed a chemically modified BSA especially for this purpose. Newsletter #1 gives in-depth information regarding it.



Immunogold Silver Staining of E-cadherin on a paraffin section of human skin. Courtesy of R. Moella, Dept. of Exp. Path., EUR, The Netherlands. • Mouse monoclonal anti E-cadherin • GAM IgG UltraSmall • Aurion R-Gent SE-LM

FREQUENTLY ASKED QUESTIONS

Are there any fora which I can address with questions regarding labeling or microscopy?

Feel free to address our HELPDESK by e-mail with questions regarding immunolabeling.

There are a few newsgroups which may be of interest: **bionet.cellbiol, bionet.cellbiol.cytonet, bionet.molbio.methds-reagnts and sci.bio.immunocytochem**. There is a microscopy listserver to which you can subscribe and which offers a platform to ask questions regarding light and electron microscopy in all its facets. You can subscribe by sending an e-mail message to **ListServer@MSA.Microscopy.Com**. The message only has to contain the words "subscribe microscopy".

How can I do a controlled silver enhancement with pre-embedding?

With pre-embedding there are 2 possibilities: either the enhancement is done before embedding or on the sections after embedding. We prefer to do the enhancement on sections (on nickel grids) since this gives more control over the degree of enhancement. Using longer enhancement times allows to observe larger (even ultra thin) sections in the light microscope. This facilitates searching for the area in the specimens where a reaction has occurred and allows easy targeting and trimming down to the area of interest for EM sectioning. Shorter enhancement is then used on sections for EM.

Using enhancement before embedding has the disadvantage that once enhancement proves to be too long (resulting in too large particles) this can not be reversed.

In which case should I use a Single Fab or F(ab')$_2$ conjugate instead of the complete immunoglobulin conjugate?

The size of a conjugate is co-responsible for its efficiency. The overall size is determined by the particle size and by the size of the proteins adsorbed onto the particle surface. That is why we introduced Ultra-Small particles in the first place. Whenever a specimen is relatively dense or intensely cross-linked immuno reagents will be more hindered in their action. If you are already using an Ultra-Small conjugate further improvement may result from using a single Fab or F(ab')$_2$ fragment of the specific secondary antibody instead of the intact Ig-molecule.

When should I use normal serum in the incubations?

It is a good idea to use normal serum as an additive to the blocking and incubation buffer when using secondary antibody conjugates. The normal serum should be the same species as the secondary antibody conjugate. Its action is similar to the action of BSA. Please be careful when using normal sera to suppress background with Protein A or Protein G conjugates. These conjugates detect several Ig-types from different species which, when used as normal serum additive, would lead to an impressive amount of gold particles all over the specimen. We offer several Blocking Solutions tailored for specific secondary antibody or protein A/G incubations.

What about sensitivity, signal-to-noise ratios and detectability?

Sensitivity can be considered at different levels in the total preparation and incubations. Ideally during preparation one would like to preserve all antigens present. In many cases this is not possible. But at least a representative fraction should be preserved and be available for immuno labeling. It all depends on the preparation procedure (fixation, embedding, temperature, etc.), which leaves you with a specimen or section with a given number of available and recognizable antigens. The ensuing detection protocol has 100% sensitivity if all the remaining antigens are detected, i.e. are represented by at least one gold particle or marker molecule. Again, due to masking and steric hindrance by the specimen composition this will only in exceptional cases by fully attained.

The immuno labeling sensitivity thus expresses the degree to which available antigens can be detected by the employed combination of primary antibody and secondary conjugate.

The quality of the primary antibody is the next important item. Theoretically the Kd-value of an antibody/antigen reaction is a measure for the dilution at which the incubations should be performed and for the stability of the ensuing bond. Sensitivity will go up with more concentrated antibody solutions up to a maximum level. However, when the primary antibody shows cross-reactivity there is not necessarily an improved signal-to-noise ratio. The reliability of the detection by the primary antibody improves in such cases with higher dilutions, probably leading to a smaller amount of antigens detected, but to an improved **signal-to-noise** ratio.

Thus, sensitivity at the level of the primary antibody has to be balanced against the signal-to-noise ratio.

The last step is the quality of the secondary reagent. In fact you will be looking at a number of gold particles which represents a number of secondary antibodies which have detected a number of primary antibodies. For the interaction between the secondary reagent and the primary antibody the same rules apply as indicated for the antigen/primary antibody reaction.

Detectability reflects the degrees to which the final result of all the reactions involved can actually be seen. This is depending on the right match between particle size and magnification. Ultra-Small particle-based conjugates for instances are among the most efficient detection systems, but you will only detect them after silver enhancement (in most applications).

What is epi-polarization microscopy?

Epi-polarization is a technique used for the very sensitive light microscopical observation of metal particles. Where bright field microscopy depends on contrast levels in discriminating signals, epi-polarization works differently: provided particles are large enough individual particles will be observed. So in fact you are evaluating your labeling results on the same basis as with an electron microscope by looking at individual particles. This makes this technique so valuable as it builds a bridge between the light level and the electron microscopical observation.

What you need to do is this: a high-quality light microscope equipped with an epi-illumination source, preferably a high pressure Hg-lamp (although a halogen source may also do). Many laboratories have an epi-fluorescence microscope at their disposition with a 40X (or higher) oil objective. Such microscope equipment forms the correct basis. You only need to implement an epi-polarization filter (the so-called epi-block or IGSS filter) in the filter housing. The epi-block contains two polarizers, differing 90 degrees in orientation with respect to each other.

How does it work (in short)? High intensity light passes the first polarizer in the epi-block and becomes polarized. The polarized incident light passes the objective lens and interacts with the specimen. The biological material hardly gives any reflection, and the reflected light is unmodified. The metal particles mirror the polarized light, thereby randomizing the polarization angle.

Reflected light passes up through the objective lens. On its way to the eye pieces or the photo camera the light passes the second polarizer in the epi-block. While doing so, light with the original polarization angle (the way it was polarized in the first place before ever hitting the specimen) is extinguished, whereas light that has become randomly polarized (and which comes from the silver metal particles) passes the epi-block. As a result you will see individual bright stars (the gold/silver particles) against a dark background.

Epi-polarization observation can be combined in real time with bright field imaging, providing for a very sensitive detection of even extremely low amounts of antigen while still having the advantage of full morphological details in the specimen.

My specimens for pre-embedding have a lot more antigens than a corresponding ultrathin section. Should I use more concentrated reagents?

The increased amount of antigens should be balanced by a larger amount of reagent volume at an appropriate dilution (the same as used on sections with low amounts of antigen), and not by more concentrated reagents. The reason is that with increased concentration more cross-reactions may occur and signal-to-noise ratios will decrease.

Incubating specimens for pre-embedding in larger volume quantities is best performed on a rocking table for a prolonged time to warrant penetration to antigenic sites in the specimen.

What is the advantage of gold nanoparticle conjugation via adsorption?

This is an easy and straight forward method for the conjugation of high molecular weight macromolecules. The flocculation test that tests the effect of a high salt concentration on the stability of the complex makes it easy to check if adsorption to the gold nanoparticle surface is successful or not.

Can you give me more information on gold nanoparticle conjugation strategy?

First check if conjugation via adsorption is feasible. If yes, check bio activity e.g., via a dot spot test. Biomolecules that are too small to be conjugated via the classic adsorption method can be covalently linked to functionalized gold nanoparticles as an alternative. A prerequisite for successful conjugation to Carboxyl-functionalized gold nanoparticles is the presence of primary amine(s) in the target molecule. As a rule of thumb conjugation via classic adsorption to conventional gold nanoparticles is possible with macromolecules having a MW > 40 kDa.

What is the conjugation principle behind covalent conjugation using carboxyl-functionalized gold nanoparticles?

The conjugation relies on the well known and proven EDC/sulfo-NHS chemistry. EDC/sulfo-NHS activation at pH 5 results in an amine reactive ester, immediately followed by binding to free amine on the target molecule.

ImmunoGold Newsletters & Flyers

Newsletter 1 *Optimized immuno labeling using AURION Blocking Solutions and AURION BSA-c™* by Jan L. M. Leunissen and P. van de Plas

Newsletter 2 replaced by Newsletter 5

Newsletter 3 *In situ hybridization for Light and Electron Microscopy using Ultra Small Gold detection and Silver Enhancement* by P. van de Plas

Newsletter 4 *The Dot-Spot test: a simple method to monitor immunoreagent reactivity and influence of fixation on antigen recognition* by Hong Yi and Jan L. M. Leumissen

Newsletter 5 *Ultra Small Gold Reagents and R-Gent SE-EM in Pre-embedding single and double labeling* by Hong Yi and Jan L.M. Leunissen

Newsletter 6 *Gold Nanoparticle conjugation: Adsorption or Covalent Binding?* by Peter van de Plas, Stan Willems, and Jan Leunissen

Newsflyer 1 *Background suppression in Immunoperoxidase Cytochemistry using AURION BSA-c™* by Wim Voorhout and Katja Teerds

Newsflyer 2 – Part 1 *Double label Immunohistochemistry for the separate observation of two antigens using Epi-polarization Microscopy for the Immunogold-Silver technique and Fluorescence Microscopy for the Alkaline Phosphatase Staining* by C.M. van der Loos

Newsflyer 2 – Part 2 *A comparison between the Streptavidin-Biotin-Alkaline Phosphatase method and the Immunogold technique using Ultra Small gold particles and silver enhancement* by R. Willemsen

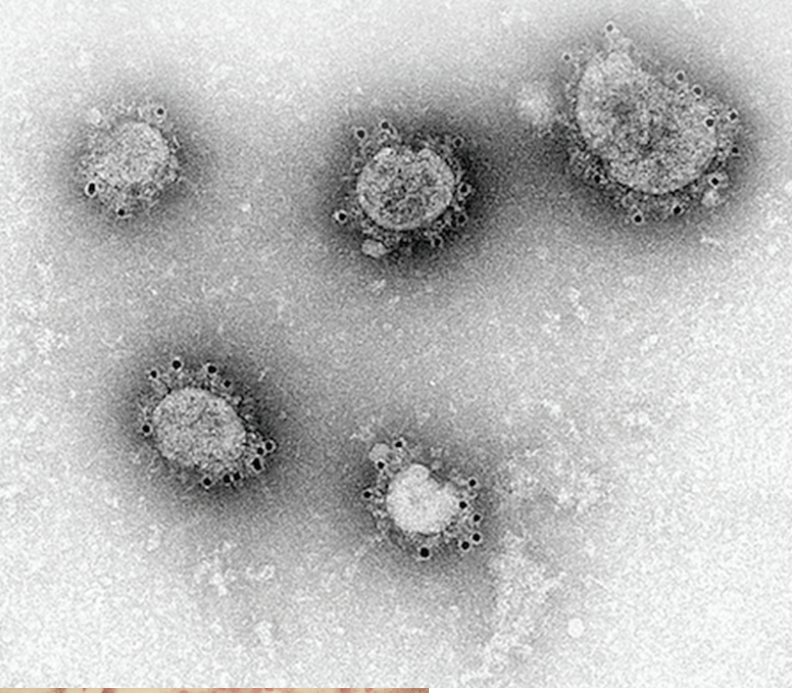
WORKSHOPS

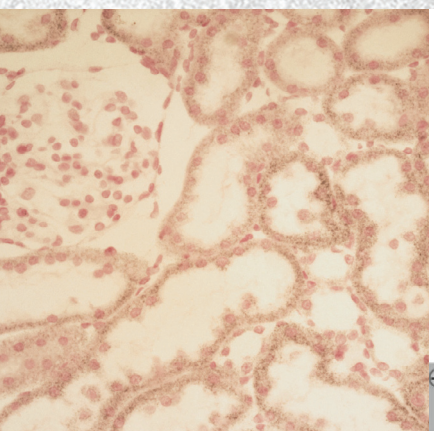
Endless Possibilities...

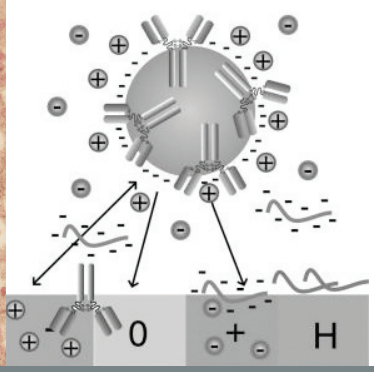
EMS Microscopy Academy

Aurion Immunogold Silver Staining Workshop

Three days of hands-on training for students, researchers, and microscopists who want to learn the most up to date theory and practice in Immuno Gold labeling.







Scope of Class

The objective of the course is to provide researchers with the opportunity to learn the theory and practice of Immuno Gold labeling. Participants will process their own samples under the expert guidance of our tutors, who are experts in Immuno Gold Silver Staining techniques.

During the workshop attendees will receive theory, including but not limited to immune detection, in situ hybridization, silver enhancement, as well as background issues. There will be time for practice as well. Attendees will be able to work with their own specimens, as well as ones we will have prepared. A full review of incubation methods, testing of antigenicity and reactivity, complete principles of Immuno Gold labeling, and preparation of conjugates for EM and LM will be covered.

Format

Lecture, demonstration and hands-on practice, as well as round table discussion. Participants may bring their own samples to work on during lab time.

Note: Specimens need to be "ready to use", e.g., sections of chemically fixed cells on coverslips. For each combination of primary antibody and gold conjugate that you want to test during the workshop, you need at least 3, preferably 4, specimens. This enables you to test different dilutions of the primary antibody and to check the Immuno Gold/Silver reagents (negative control).

For pre-embedment immuno labeling, also include specimens that will be immuno labeled for evaluation on the light microscope. These specimens are used to see if the experiment is successful. During the workshop there is no time/no options to further process pre-embedment specimens for EM evaluation. Plastic embedment and sectioning needs to be done at your own facility.

Main Curriculum

- Gold nanoparticle conjugation
- Theory underlying Immunogold labeling protocols
- Silver enhancement of gold particles
- Immunogold labeling on a variety of sample preparations of LM
- Immunogold labeling for EM
  - Pre-embedment immunogold labeling using ultra small gold conjugates and silver enhancement
  - Post-embedment immunogold labeling on plastic and ToKuyasu cryo sections using conventional colloidal conjugates and ultra small gold conjugates
  - Manual and automated immunogold labeling
- Pre/Post-embedment double Immunogold labeling
- Background minimization in Immunogold labeling
- Troubleshooting

Facility

The EMS Microscopy Academy: Located in Hatfield, Pennsylvania, the Academy provides electron microscopy classes, workshops and training sessions for all fields of microscopy, including materials science and biological science.

Find out more or register at:

www.emsmicroscopyacademy.com

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We hope to see you there!

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