Introducing...

UranyLess

EM STAIN

A Substitute for Uranyl Acetate
EMS is proud to introduce UranyLess, a new contrast stain solution for TEM, for all of your negative staining applications. It is an amazing substitute for Uranyl Acetate with similar results.

After only a minute of contact, UranyLess' fast-acting, non-radioactive lanthanide mix is finished staining your sections or deposits (see protocols below). If needed, lead citrate is recommended to increase the contrast. UranyLess is non-diffusible and is at pH 8.2. The 30ml airless bottle will take approximately 1500 grids. The airless bottle increases the shelf life, eliminates CO₂ contamination, and produces less waste — the solution pumps out in perfect amounts without leaking or spilling. UranyLess is also available in a larger amount for use in automated staining equipment. When using UranyLess for automated staining, do not wash longer than 10 minutes or you run the risk of losing all contrast.

UranyLess has been tested on many biological tissue (animal and plant): intestine, skeletal and cardiac muscle, liver, kidney, adrenal gland, nerve, cell culture, plant tissue, and also on negative staining of bacteriophage, bacteria, and polymers. UranyLess is ideal because of its ability to stain any kind of material and results are reproducible.

**PROTOCOLS OF USE**

**Classic Contrast**

This protocol is used for double staining with UranyLess/Lead citrate on ultrathin sections. This protocol is adapted to biologic samples that have been fixed with glutaraldehyde, osmium, or ruthenium and embedded in an epoxy type resin (Epon, Araldite, Spurr) or acrylic type (LRWhite, HM20).

**Staining Protocol:**

1. Place a drop of UranyLess on parafilm or any other hydrophobic slide.
2. Place the grid on the UranyLess drop for 1 to 2 minutes.
3. Blot the grid on a filter paper and then wash in distilled water.
4. Let it dry.
5. After drying, go to the lead citrate staining according to Reynolds method (1963).
6. Place the grid on the lead citrate drop according to the Reynolds method, for 1 minute.
7. Blot the grid on a filter paper before rinsing with distilled water.
8. Let it dry.

**References**


**Ordering Information**

- 22409 UranyLess EM Stain* 30 ml
- 22409-20 UranyLess EM Stain 200 ml

* in airless bottle

**Technical Tip:**

UranyLess is not air or light sensitive, unlike Uranyl Acetate. After lead citrate, place the grid in a 100% propylene glycol wash bath or for 5 minutes.

**Negative Staining**

Negative staining is a very useful technique in electron microscopy. It allows characterization of isolated particles of morphology as bacteria, virus, protein, nanoparticles, liposomes, exosomes, etc.

**Staining Protocol:**

1. On a piece of parafilm or any other hydrophobic carrier, place a drop of your solution (~10µl) and a UranyLess drop.
2. Using our fine tweezers, place your sample drop on a formvar-carbon coated grid for about 1 minute.
4. Place your grid on the UranyLess solution for 1 minute.
5. Blot, let it dry for 5 minutes and observe under the microscope.

**Technical Tip:**

UranyLess is not air or light sensitive, unlike Uranyl Acetate. Do not wash longer than 10 minutes or you run the risk of losing all contrast.

**Negative Staining**

Negative staining is a very useful technique in electron microscopy. It allows characterization of isolated particles of morphology as bacteria, virus, protein, nanoparticles, liposomes, exosomes, etc.

**Staining Protocol:**

1. Place a drop of bacteria or any other hydrophobic particle, place a drop of your solution on a formvar-carbon coated grid.
2. Drain off excess liquid, place your sample drop on a formvar-carbomedici sputter grid.
3. Drain off excess liquid, place your grid on the grid in the grid for 1 minute.
4. Blot, let it dry for 5 minutes and observe under the microscope.
### Polymersomes

UranyLess was tested in comparison with uranyl acetate, which is at acidic pH 4 (seems to disrupt the organization of the molecular structure) in comparison also the comments by the technique Cryo SEM (scanning electron microscopy).

The chemical structure is organized as follows:

Polymersome, Observation

**Microscopy Scanning in Freeze Mode.** Photo: The Toulouse Laboratory IMRCP, team Anne-Françoise Mingotaud.

Polymersomes, Negative Staining in Uranyl Acetate pH 4. Photo: The Toulouse Laboratory IMRCP, team Anne-Françoise Mingotaud.

Polymersomes, Negative Staining 1 mn in UranyLess in pH 7 Aqueous Solution. Photo: The Toulouse Laboratory IMRCP, team Anne-Françoise Mingotaud.

### Yeasts

Preparation of the sample using the following protocol:

- Classic Fixation: Glutaraldehyde - Osmium - Included in Epon
- Contrast the UranyLess monitoring Lead Citrate

Yeast. Photo: Jeannine Lherminier (INRA - Dijon).

### Trematodes

Preparation of the sample using the following protocol:

- Classic Glutaraldehyde Fixation, Osmium, Inclusion in Spurr Resin
- Contrast the UranyLess monitoring Lead Citrate

Trematodes. Photo: Yann Quilichini (Microscopy Platform of the University of Corsica - Corte)

### Polymersomes

Vesicles were tested in contrast with uranyl acetate, which is at acidic pH 4 (seems to disrupt the organization of the molecular structure) in comparison also the comments by the technique Cryo SEM (scanning electron microscopy).

The chemical structure is organized as follows:

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YS112200521.png

YS112200521.png

### FREQUENTLY ASKED QUESTIONS

#### What is UranyLess made from?
UranyLess is a solution ready for use, a mix of lanthanides (rare-earths).

#### How is UranyLess sold?
In an aqueous solution (water).

#### What is its shelf life?
One year.

#### What are the storage conditions for UranyLess?
Store it at room temperature away from direct sunlight.

#### Does it need to be diluted?
No, it is sold ready for use.

#### What is its pH?
UranyLess pH is 6.8–7.

#### How to stain with UranyLess?
Simply drop UranyLess on your grid, and wait a minute. Dry, then contrast with lead citrate according to Reynolds method.

#### Is it the same protocol for every kind of tissue (animal, plant, marine)?
Yes it is - a double stain of UranyLess plus Lead citrate.

#### Does it adjust to every kind of resin?
Yes, it operates with every kind of resin (Epon, Araldite, Spurr).

#### Can it be used on negative staining?
Yes, it can be used on negative staining.

#### Can it be used for other substrates?
Some tests are in progress.

#### Is it adaptable to a cryo-stain?
No, because it is prepared in water. However, we are currently developing many formulations of UranyLess, including ethyl UranyLess and acetone UranyLess, the latter being the most adapted to cryo-genic use.

#### Is it efficient on marine material?
Yes.

#### How is UranyLess packaged?
We sell UranyLess in an airless 30ml bottle and also in a brown 200ml bottle.

#### Can it be used with automated staining equipment?
Yes, the 200ml bottle is available for use with automated staining equipment. When using UranyLess for automated staining, do not wash longer than 10 minutes or you run the risk of losing all contrast.

### UranyLess Applications

All photos taken on Transmission Electron Microscope.
Reconstituted Epidermis
Preparation of the sample using the following protocol:
• Fixing: Classic Glutaraldehyde, Osmium, Epon / Araldite
• Cutting: Ultra Fine, Double Contrast and Lead Citrate

Muscle - Nerve - Mice
Preparation of the sample using the following protocol:
• Fixing: Classic Glutaraldehyde, Fixation PFA, Osmium, Epon
• Cutting: Ultra Fine, Double Contrast and Lead Citrate

Mouse Ovarian Follicle
Preparation of the sample using the following protocol:
• Fixing: Classic Glutaraldehyde, Fixation PFA, Osmium, Epon
• Cutting: Ultra Fine, Double Contrast and Lead Citrate

Mouse Kidney
Preparation of the sample using the following protocol:
• Fixing: Classic Glutaraldehyde, Osmium, Epon
• Cutting: Ultra Fine, Double Contrast and Lead Citrate

Mouse Cardiac Muscle
Preparation of the sample using the following protocol:
• Fixing: Classic Glutaraldehyde, Osmium, Epon
• Cutting: Ultra Fine, Double Contrast and Lead Citrate
Uran yLess Applications

All photos taken on Transmission Electron Microscope.

Plant Tissue
Preparation of the sample using the following protocol:
• Glutaraldehyde Fixation
• Contrast the UranyLess monitoring Lead Citrate

Drosophila Larva
Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation, Epon-embedded
• UranyLess Lead - Citrate

Phage T6
Preparation of the sample using the following protocol:
• Staggering Phage T6 on a 300–Cu grid
• Post Coloring of 1 minute

Cross-Sectional Bacteria
Preparation of the sample using the following protocol:
• Fixing Classic Glutaraldehyde
• Cutting Ultrafine, Double Contrast UranyLess and Lead Citrate

Sacculina Crustaceans (Small Parasitic Crustacean)
Preparation of the sample using the following protocol:
• Classic Glutaraldehyde Fixation, Epon, UranyLess Inclusion
• Fine Cups - Contrast to the Aqueous UranyLess to 60°C on a Hotplate without Lead Citrate Post Coloring

Liver Mouse and Gerbil Sahara
Preparation of the sample using the following protocol:
• Glutaraldehyde Fixation, Epon
• UranyLess Lead - Citrate

Adrenal Gland
Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation, Epon
• UranyLess Lead - Citrate

Bacteria E. Coli
Preparation of the sample using the following protocol:
• Fixing Classic Glutaraldehyde
• Cutting Ultrafine, Double Contrast UranyLess and Lead Citrate

Intestine
Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation, Epon
• UranyLess Lead - Citrate

Phage T6
Preparation of the sample using the following protocol:
• Staggering Phage T6 on a 300–Cu grid
• Post Coloring of 1 minute

Adrenal Gland
Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation, Epon
• UranyLess Lead - Citrate

Sacculina Crustaceans (Small Parasitic Crustacean)
Preparation of the sample using the following protocol:
• Classic Glutaraldehyde Fixation, Epon, UranyLess Inclusion
• Fine Cups - Contrast to the Aqueous UranyLess to 60°C on a Hotplate without Lead Citrate Post Coloring

Liver Mouse and Gerbil Sahara
Preparation of the sample using the following protocol:
• Glutaraldehyde Fixation, Epon
• UranyLess Lead - Citrate

Adrenal Gland
Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation, Epon
• UranyLess Lead - Citrate
Parsley and Rosebush, Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
• Ultrafine Cup - Contrast UranyLess lead -citrate

Culture Cells, Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
• Ultrafine Cup - Contrast UranyLess lead -citrate

PLC Contrast Leica EM Stain
Preparation of the sample using the following protocol:
• Classic-Glutaraldehyde Fixation PFA, Osmium, Epon
• Ultrafine Cup - Contrast UranyLess lead -citrate

All photos taken on Transmission Electron Microscope. 
We present here some images made by Hitachi transmission electron microscope with a digital camera AMT. You will notice that the combined action of potassium ferrocyanide and UranyLess reveal a marked way the cyto-membranes in the ileum.