

### Introduction

Colloidal gold particles are readily visualized in the QX-302 capsules. Gold beads conjugated to a variety of molecules, such as Protein A, Immunoglobulins and Streptavidin, are commercially available and can be used to immunolabel specific antigens.

#### ➤ The reagents required:

- PBS
- 1% BSA in PBS, pH 8.2
- Unmasking reagent (for example Auto/Zyme<sup>®</sup>, Biomedica Corp., Cat. No. M34)
- Blocking agent (BSA, normal Serum or other)
- Primary antibody
- Gold particle conjugate
- Double distilled water
- Silver enhancement kit (for example, AURION R-GENT SE-EM, Cat. No. 5000.033)
- Tween<sup>®</sup> 20 (for example Sigma Cat. No. P-7949)

#### ➤ Procedure:

1. Wash three times with PBS, 5 minutes each wash.
2. If necessary, use an unmasking reagent (for example, Auto/Zyme<sup>®</sup>) in accordance with manufacturer's recommendations, then wash three times with PBS, 5 minutes each wash.
3. To avoid non-specific background, incubate with a blocking solution, such as 1% BSA and 5% normal Serum from the species of the secondary antibody in PBS for 30 minutes.
4. Incubate with primary antibody in 1% BSA in PBS. Carry out a control reaction without the primary antibody.



#### NOTES

- a. *Carry out and test serial dilutions to determine the optimal concentration of the primary antibody*
- b. *An incubation period of one to two hours at room temperature usually renders good results.*
- c. *For some antibodies, labeling can be improved by incubating at 37°C or by longer incubation periods (several hours to overnight) at 4°C.*

# QUANTOMiX™

## Protocol for Sample Preparation (QX-302)

Doc. No.: QXP011	V 1.0	<b>Title: Immunogold Labeling of Tissues</b>	
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5. Wash three times with 1% BSA in PBS, pH 8.2, 5 minutes each wash.

**NOTE**

*In case of background problems, a mild detergent such as 0.05% to 0.1% Tween® 20 can be added to the wash buffer.*

6. Incubate with the gold-labeled secondary agent (for example gold conjugated secondary antibody or Protein A or G) in protein containing solution, such as 1% BSA in PBS, pH 8.2.

**NOTE**

*For optimal dilution and conditions, refer to the manufacturer's recommendations.*

7. Wash three times with 1% BSA in PBS, pH 8.2, 5 minutes each wash.
8. Wash three times with double distilled water, 5 minutes each wash.
9. If necessary, perform silver enhancement.

**NOTE**

*The AURION R-GENT SE-EM kit is recommended. However, other comparable kits are also available.*

10. Wash four times with double distilled water, 5 minutes each wash.

**NOTE**

*Protocols provided by QuantomiX are based on work conducted at QuantomiX laboratories. They are given as a starting point which will facilitate the user's first steps in acquiring the desired imaging results. It is the user's responsibility to determine the suitability of any protocol published by Quantomix to their applications. Users may find it necessary to modify protocols in order to obtain the information required for their study.*