

Introduction

Colloidal gold particles are readily visualized in the QX-102 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
- Fixative
- 0.2% Triton® X-100 in PBS (for intracellular antigens)
- Blocking agent (normal Serum or other)
- Primary antibody
- Wash buffer (1% BSA in PBS, pH 8.2)
- Gold particle conjugate
- Distilled water
- Optional - Silver staining kit (for example, AURION R-GENT SE-EM, Cat. No. 5000.033)
- Optional - Gold staining kit (for example, Nanoprobes Gold enhance EM, Cat. No. 2113)

➤ Procedure:

1. Wash four times with PBS.
2. Fix the cells, referring to fixation protocols.
3. Wash four times with PBS.
4. Permeabilization for intracellular antigens:
 - a. For Paraformaldehyde or Glutaraldehyde fixed cells, permeabilize the cells by incubating with 0.2% Triton® X-100 in PBS for 10 minutes.
 - b. For Methanol fixed cells, no additional permeabilization is required.
 - c. Wash twice, 5 minutes each wash, with PBS.
5. To avoid non-specific background, incubate with a blocking solution (for example 1% BSA and 5% normal serum from the species of the secondary antibody in PBS, pH 8.2) for 30 minutes.
6. Incubate with primary antibody, diluted in 1% BSA in PBS, pH 8.2. Carry out the control reaction without the primary antibody.



NOTES

- a. *Carry out and test serial solutions to determine the optimal concentration of the antibody*
- b. *An incubation period of 30 to 60 minutes 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.

7. Wash four times with 1% BSA in PBS, pH 8.2.



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.

8. Incubate with the gold-labeled secondary agent (gold conjugated secondary antibody or Protein A or G) in protein containing solution, such as 1% BSA in PBS, pH 8.2 or 5% normal Serum.



NOTE

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

9. Wash four times with 1% BSA in PBS, pH 8.2 to remove unbounded antibodies.
10. Wash twice with PBS.



NOTE

It is possible to post-fix the sample with 2% glutaraldehyde for 5 minutes, than wash twice with PBS.

It is not recommended to post-fix the sample when silver enhancement is performed.

11. If silver enhancement is required, complete steps 13-14 and then step 17.
12. If gold enhancement is required, complete steps 15-17.
13. Wash six times; 5 minutes each wash, with double distilled water.



NOTE

a. The AURION R-GENT SE-EM kit is recommended for silver enhancement. However, other comparable kits are also available.

b. The silver enhancement incubation time has to be optimized according to the detection limits of the SEM to be used.

14. Incubate with a freshly prepared silver enhancement solution. The incubation time depends on the original gold bead size and the required final particle size. Refer to the manufacturer's recommendations.
15. Wash ten times with double distilled water.
16. Incubate with a freshly prepared gold enhancement solution. The incubation time depends on the original gold bead size and the required final particle size. Refer to the manufacturer's recommendations.
17. Wash six to ten times with double distilled water.



Protocol for Sample Preparation (QX-102)

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NOTE

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