AURION ULTRA-SMALL GOLD REAGENTS

PRODUCT INFORMATION

AURION Immuno Gold Reagents are prepared using the highest quality antibodies or binding agents available. All antibodies are immuno affinity purified and immuno cross-adsorbed to diminish and frequently prevent non-specific reactions.

AURION ULTRA-SMALL GOLD REAGENTS and AURION R-GENT silver enhancement reagents are recommended for facilitated penetration when localizing intracellular antigens and for increased sensitivity, both for light and electron microscopy and for bio-assays.
AURION ULTRA-SMALL GOLD REAGENTS are supplied in PBS with 1% Bovine Serum Albumin and 15 mM NaN_3.

AURION ULTRA-SMALL GOLD REAGENTS are tailored to contain 60-80 µg of specific protein/ml. The average gold cluster diameter is below 0.8 nm.

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.

AURION 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.
Do not freeze!

MARKING FOR LIGHT MICROSCOPY

AURION ULTRA-SMALL GOLD REAGENTS are adequate for the localization of extracellular and intracellular antigens in light microscopy.

WHOLE MOUNT PREPARATIONS

Living cells are preferably incubated at 0-4°C or in the presence of 0.05-0.2% NaN_3 in order to prevent internalization of reagents.
Monolayers on coverslips are easily incubated using 6-well culture plates (Falcon, Nunc etc.) The glass coverslips are placed in the well and covered with incubation media (approximately 100 µl). During washing the coverslips are covered with 2 ml of washing medium and left on a rocking table.
Cell suspensions are gently pelleted after each incubation step. The pellets are resuspended in the medium used in the next step and the centrifuge tube containing the suspension is left on a rocking table.
On occasion efficient background suppression is obtained by using 1-10% heat inactivated Human AB-serum as additive to the incubation buffer.

The degree of penetration of immuno reagents into the cell interior depends on size of the reagents, specimen characteristics and (aldehyde)fixation. Single Fab Ultra-Small Gold reagents are available for optimal penetration (see auxiliary products).

For the localization of intracellular antigens AURION ULTRA-SMALL GOLD REAGENTS **occasionally** require a permeabilization step.

PERMEABILIZATION PROCEDURE:

Note: The use of more or less apolar fixatives (e.g. based on methanol, acetone, ethanol) already infers a limited degree of permeabilization to specimens as part of the lipid is removed.

Whenever a permeabilization step should be necessary the following procedure may be employed:
Triton-X-100® is added in a final concentration of 0.1 - 0.5% to the washing buffer used only immediately after fixation. This first washing step should last between 10 and 20 minutes while shaking gently.
Whereas this procedure is suited for light microscopical applications, the ultrastructural preservation is in many cases not adequate for electron microscopy.

A more gentle permeabilization method is indicated below:
Permeabilize the specimens by dehydration after fixation in a graded series of ethanol in distilled water (50, 70, 90, 2x100% for at least 1 minute each). As a final step the specimens are rinsed in acetone 100% for at least 5 minutes while whirling. Specimens are rehydrated using the graded ethanol series in reversed order. Each step should last about 2 minutes at least.

SECTION LABELING

When used on paraffin, vibratome or cryostat sections the use of permeabilization procedures is optional as well as protein digestion treatment or antigen retrieval. Specimens are preferably washed in 250ml staining trays with separate insert, on a magnetic stirrer.

MARKING FOR ELECTRON MICROSCOPY

PRE-EMBEDDING SET-UP

The set-up for marking whole mount preparations in light microscopy is used. Single Fab Ultra-Small Gold reagents are available for optimal penetration (see auxiliary products).

INCUBATION SET-UP FOR ON-GRID MARKING

The use of nickel grids is recommended, especially since silver enhancement procedures are intended to be used.
For most applications grids are floated on top of drops of immune reagents displayed on a sheet of parafilm. They are washed on larger drops of buffer. Whenever larger series of grids or coated grids need to be processed, the use of microtiter plates is preferred during incubations to avoid the risk of cross-contamination (e.g. Falcon 3034, Falcon Plastics, Oxnard, CA 93030, USA).
Transfer of the grids from droplet to droplet or from well to well can be performed with fine forceps or a nickel coated metal loop.

MARKING FOR BIO ASSAYS

Depending on size and type of substratum the incubations can be performed in sealed plastic bags, Petri dishes or in disposable screw cap sealed tubes.

INCUBATION BUFFER SYSTEM

PBS, (20 mM Phosphate buffer, 150 mM NaCl), pH 7.4
0.1-0.2%AURION BSA-c ™
15 mM NaN_3
check the pH and adjust to 7.4 if necessary

This buffer system is recommended both for secondary antibody reagents as well as for streptavidin reagents.

Note on background prevention:

A special AURION NEWSLETTER dealing with the topic of background is available on request.

As an alternative, the following incubation buffer system may be used: PBS (10mM Phosphate buffer, 150mM NaCl), pH 7.6 supplemented with
0.8% Bovine Serum Albumin and
0.1% Cold Water Fish Skin Gelatin
15 mM NaN_3
check the pH and adjust to 7.6 if necessary
The effectiveness depends on specimen and antibody characteristics and has to be tested. The use of cold water fish skin gelatin has been recommended by Behnke et al., *J. Cell Biol*., 41, (1986), 326.

Buffers are either prepared immediately before use or thawed from aliquots stored at -20°C.

ACTUAL PROCEDURE

1 To inactivate residual aldehyde groups present after aldehyde fixation specimens are incubated with 0.05 M glycine or lysine in PBS buffer for 10-20 minutes.

   For whole mount / pre-embedding applications a solution of 0.1 % $NaBH_4$ (freshly prepared from dry $NaBH_4$ stored in a desiccator) in PBS has to be used for 10 minutes. Before proceeding rinse in PBS, 2 x 1 minute.

2 FOR LIGHT AND ELECTRON MICROSCOPY
   Block with PBS supplemented with 5% BSA, 5% normal serum (same species as the antibody in the second immuno incubation step) and 0.1% cold water fish skin gelatin for 15-30 minutes. This block buffer is also available as ready-to-use solution from Aurion (see auxiliary products).

   FOR BIO ASSAYS
   Block with 5% BSA in PBS for 15-30 minutes at 45°C.

3 Wash in incubation buffer, 2 x 5 minutes.

4 Specimens are incubated as indicated in a dilution of specific primary antibody, preferably affinity-purified, 1-5 µg/ml, or a high dilution of a high titer antiserum, made up in incubation buffer for 30 minutes to 1 hour (for whole mount / pre-embedding applications incubation time has to be increased to at least 2 hours).
   Antibody concentration and incubation time may have to be adapted according to the specific characteristics of the primary antibody

If longer incubation times are required (e.g. with low titer antibody solutions) the procedure should be carried out at 4°C overnight.

5 Wash with incubation buffer for 3 x 5 minutes. Washing should be extended to 6 x 5 minutes for EM on-grid marking.

FOR STREPTAVIDIN REAGENTS IN A 3-STEP SYSTEM: Incubate with the biotinylated secondary antibody according to step 4, rinse according to step 5 and proceed with step 6.

6 Incubate with the appropriate gold conjugate reagent, diluted 1/50-1/200 in incubation buffer for 2 hours (for whole mount / pre-embedding applications incubation time has to be increased to at least 4 hours). It is recommended to test a series of dilutions for each new localization study.

7 Wash with incubation buffer for 4 x 5 minutes while aggitating. Washing should be extended to 6 x 5 minutes for EM on-grid marking.

8 Wash twice with PBS for 5 minutes each, postfix in 2% glutaraldehyde in PBS for 5-10 minutes and finally wash with distilled water 3 x 5 minutes.

9 Proceed with silver enhancement using:
   AURION R-GENT SE-LM for light microscopy and bio-assays or
   AURION R-GENT SE-EM for electron microscopy applications (see auxiliary products).

AUXILIARY PRODUCTS

CODE	DESCRIPTION
905.001	Basic blocking solution, 30ml
905.002	Blocking solution for Goat gold conj., 30ml
905.003	Blocking solution for Rabbit Gold conj., 30ml
905.004	Blocking solution for Sheep gold conj., 30ml
905.005	Blocking solution for Donkey gold conj., 30ml
900.011	Bovine serum albumin fraction V, 25g
900.033	Cold Water Fish Skin Gelatin (40%), 10ml
900.066	Normal Rabbit Serum, 5ml
900.077	Normal Goat Serum, 5ml
900.111	Normal Sheep Serum, 5ml
900.122	Normal Donkey serum, 5ml
900.022	AURION BSA-c™ (10%), 100ml
900.099	AURION BSA-c™ (10%), 30ml
100.255	Fab Goat-anti-Rabbit IgG Ultra-Small, 1.5ml
800.255	Fab Goat-anti-Rabbit IgG Ultra-Small, 0.6ml
100.266	Fab Goat-anti Mouse IgG Ultra-Small, 1.5ml
800.266	Fab Goat-anti-Mouse IgG Ultra-Small, 0.6ml
100.277	Fab Goat-anti-Rat IgG Ultra-Small, 1.5ml
800.277	Fab Goat-anti-Rat IgG Ultra-Small, 0.6ml
500.011	AURION R-GENT SE-LM, 60ml
500.022	AURION R-GENT SE-LM, 250ml
500.033	AURION R-GENT SE-EM, 30ml
500.044	AURION R-GENT SE-EM, 90ml

AURION
Immuno Gold Reagents & Accessories
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