AURION CONVENTIONAL GOLD REAGENTS

APPLICATION AREA: ELECTRON MICROSCOPY

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 CONVENTIONAL GOLD REAGENTS are based on the strictly defined particle sizes of 6, 10,15 or 25nm and are recommended for use in electron microscopy. The sols used to prepare AURION CONVENTIONAL GOLD REAGENTS are monodisperse, with a coefficient of variance <15%, but in most cases <10%.

The choice of sizes provides for multiple labelling possibilities with the least possible overlap.
The special process of production allows the small particle size of 6 nm to be used in stead of 5 nm with the same sensitivity but increased visibility in the standard transmission electron microscope (see also van Bergen en Henegouwen & Leunissen, *Histochemistry*, 85, (1986), 81.
The reagents are supplied in PBS with 1 % Bovine Serum Albumin and 15 mM NaN_3. The OD_{520nm} is adjusted to 1.0 so as to prevent aggregate formation during storage.

The actual lot specifications (size, variation and expiry date) are indicated on a separate Quality Control Sheet. 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!

REMARKS WITH RESPECT TO ULTRATHIN CRYOSECTIONS

After transfer of sections to a (nickel) grid first the covering sucrose layer has to be removed. If immuno incubations are performed immediately after sectioning the grids are simply rinsed on a drop of the incubation buffer. Mostly, however, grids are collected on a solidified 2% gelatine layer in buffer (corresponding to the incubation buffer) in a small Petri dish on ice. The sucrose layer which is facing the solidified gelatine is in this way allowed to diffuse gently away and to be at least partly replaced by incubation buffer. This procedure is supposed to be less destructive to the ultrastructure since concentration shocks are avoided. In this way sufficient grids can be collected and stored at 4°C if necessary overnight.
If the grids with the sections were stored on a gelatine layer the closed Petri dish is warmed to 37°C for 30 minutes in order to liquefy the gelatine. Incubation buffer (37°C) is added in a 1:1 ratio to the liquefied gelatine.

ON-GRID MARKING FOR ELECTRON MICROSCOPY

INCUBATION SET-UP:

The use of nickel grids is recommended, especially if silver enhancement procedures are intended.

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 flattened nickel coated metal loop.

INCUBATION BUFFER SYSTEM:

PBS, (10 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 protein A, protein G and streptavidin reagents.

Note on background prevention:

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

PBS supplemented with 0.1-0.2% Bovine Serum Albumin might already be adequate in eliminating background staining. This depends on specimen and antibody characteristics and has to be tested. The use of cold water fish skin gelatin has been recommended (Behnke et al., Eur. 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 grids are incubated on 0.05 M Glycine or lysine in PBS buffer for 10-20 minutes.

2 (FOR SECONDARY ANTIBODY AND STREPTAVIDIN REAGENTS).
Transfer the grids onto drops PBS supplemented with 5% BSA, 5% normal serum (same species as the antibody in the second immuno incubation step) and 0.1% cold water skin gelatin. This block buffer is also available as ready-to-use solution from Aurion (see auxiliary products).

(FOR PROTEIN A or PROTEIN G REAGENTS)
Transfer the grids onto drops of 5% BSA, 0.1% cold water fish skin gelatin for 15 minutes. This block buffer is also available as ready-to-use solution from Aurion (see auxiliary products).

3 Wash in incubation buffer, 2 x 5 minutes.

4 The grids are transferred onto drops of a dilution of specific primary antibody, preferably affinity-purified, 1-5 µg/ml, or a high dilution of a high titre antiserum, made up in incubation buffer for 30 minutes to 1 hour.
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 titre antibody solutions) the procedure should be carried out at 4°C overnight.

5 The grids are washed on drops of incubation buffer for 6 x 5 minutes.

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 The grids are transferred to drops of the appropriate gold conjugate reagent, diluted 1/20-1/40 in incubation buffer for 30 minutes to 2 hours. It is recommended to test a series of dilutions for each new localisation study.

7 The grids are washed on drops of incubation buffer for 6 x 5 minutes.

8 The grids are washed for 2 x 5 minutes on PBS and postfixed in 2% glutaraldehyde in PBS for 5 minutes.

9 The grids are washed on distilled water for 2 x 5 minutes and contrasted.

DOUBLE LABELING:

For double marking using secondary antibody gold conjugates, two primary antibodies produced in two different animal species are mixed and applied simultaneously (step 4).
After the washing step, a mixture of the corresponding gold conjugated reagents with two non-overlapping sizes is applied (step 6).
For double marking using protein A or protein G gold conjugates each labelling is worked out separately.
An incubation with free protein-A or protein-G for 10-20 minutes is inserted after the first gold reagent incubation at a concentration of 20-100 µg/ml. Steps 4 through 6 are then repeated using a different size gold reagent.

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

AURION
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