GE Healthcare

# Amersham AuroDye™ forte

Colloidal gold reagent for the staining of protein blots

## Product Booklet

Code: RPN490

# Page finder

# 1. Legal



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# 2. Handling

## 2.1. Safety warnings and precautions

**Warning:** ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Note that the protocol may require the use of Tris, glycine and methanol. Please follow the manufacturers' safety data sheets relating to the safe handling and use of these materials.

# 3. Product information

AuroDye forte is a stabilized colloidal gold sol, adjusted to a pH of about 3. At this low pH, the negatively charged gold particles bind very selectively to proteins by hydrophobic and ionic interactions. Due to the optical characteristics of the gold particles, AuroDye forte stains transferred proteins dark red. The sensitivity is at least comparable to the silver staining of proteins in polyacrylamide gels. It has been shown to detect many more spots in transfers of 2D-gels than silver staining in the gel (1). AuroDye forte is intended to be used on nitrocellulose and polyvinylidene difluoride (PVDF) membranes. AuroDye forte cannot be used on nylon-based membranes for total protein staining. AuroDye forte is supplied as a kit, sufficient for about 15 standard sheets (10 x 15 cm, 30 ml/sheet). It contains 500 ml AuroDye forte reagent and 10 ml Tween-20™. AuroDye forte is ready for use and should not be diluted. It is to be stored at 2–8°C. Do not freeze. AuroDye forte has a guaranteed shelf-life of one year from the date of analysis.

# 4. Procedure for use

## 4.1. Materials

- High quality chemicals; it is recommended to use deionized glass distilled ultrafiltered water.
- Filter paper Hybond™-ECL (RPN2020D or RPN82D), Schleicher & Schüll (No. 604A-24343) or Whatman 3 MM (No. 3030917); other brands of filter paper should be checked for the presence of substances that are released during the transfer and which produce a very speckled background.
- Phosphate-buffered saline 10 mM, pH 7.2; dissolve the following in 1000 ml: 8 g NaCl, 0.2g KC1, 1.44 g $Na_2HP0_4$.2 $H_20$ and 0.2 g $KH_2P0_4$.
- Tween-20; not all brands are satisfactory, therefore a quality-controlled Tween-20 is included in the kit.

## 4.2. Remarks on the electrophoresis and electro-transfer procedure

The high sensitivity of AuroDye forte, used in the protein staining procedures requires special care and handling to avoid background staining. High quality chemicals should be used throughout.

Many researchers routinely work with far less sensitive stains, such as Amido black, or with relatively insensitive overlay detection techniques, which generally require heavy protein loads.

Heavy protein loads on gels, when transferred to a membrane, will not only be heavily stained, but leak protein off the membrane from saturated sites. Excessive protein leakage will cause an aggregation of the gold particles and destroy the AuroDye forte reagent. The problem will be more severe with 1D-gels than with 2D-gels, where the proteins are distributed as spots over the blotting membrane. In

general, for 1D-gels it is recommended to use protein loads which, in the case of a complex mixture, gives resolvable banding patterns after silver staining. Single bands in 1D-gels should not exceed 1000 ng/band. The ideal load for molecular weight standards is 200 ng/band. Lower loads lead to better separations and help save precious samples. For immunovisualization, AuroDye forte is best used in conjunction with a detection system, such as the AuroProbe™ BLplus (RPN 460-67) enhanced with IntenSE™BL (RPN 492) for immunogold silver staining (IGSS), which matches its high sensitivity.

It is important to wear gloves when handling gels and blots. For extra care, handle blots by their edges with forceps, as gloves can leave smears. All the current laboratory electrophoresis protocols may be used. 2D-gels should be thoroughly washed in several changes of excess transfer-buffer, in order to remove the ampholytes.

In a ID-gel system, blots contain one to several lanes. Identical blots can react in parallel with various probing agents (for example, antibodies). Usually one blot with an additional lane containing molecular weight standards is stained with AuroDye forte.

Low ionic strength transfer buffers are recommended for blotting (for example, 25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3). When SDS is used in the transfer buffer to increase transfer efficiency, the following procedure is recommended. An initial transfer is done overnight at 4V/cm with initial transfer buffer (25 mM Tris, 192 mM glycine, 0.05% SDS, 20% methanol, pH 8.3). The initial transfer buffer is then removed (can be reused once) and the apparatus, cassettes and cooling coils are rinsed with distilled water. The apparatus is filled with secondary transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3) and the transfer continued at 4V/cm at 2–8°C for 2 hours to remove SDS from the membrane.

# 5. Sandwich set-up

To obtain optimal protein staining with minimal background, it is important to use the following sandwich set-up for electro-transfer:

- the cathode-oriented grid
- a Scotch-Brite™ pad
- 2 sheets of filter paper
- a transfer membrane
- the gel
- a transfer membrane
- 2 sheets of filter paper
- Scotch-Brite pad
- the anode-oriented grid (see Figure 1)

We found that the use of an extra transfer membrane at the cathodic side of the gel and the use of a high quality filter paper lead to sharp, high contrast staining with negligible background. Wash the Scotch-brite pads extensively with distilled water after each run to remove protein contaminants.

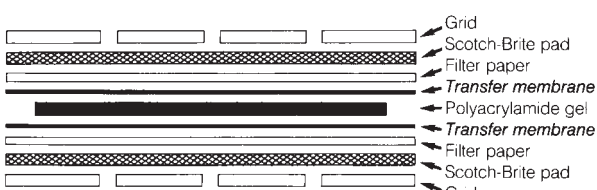


**Figure 1:** Schematic representation of the protein blotting sandwich set-up.

Several companies now offer semi-dry blotters. This new system allows highly efficient transfer in a short time period (1 hour) and consumes negligible amounts of buffer. No special high current power supply is necessary. Since only negligible heat is produced, cooling is not necessary. An extra blotting membrane is not needed.

If a slightly higher signal is required with a clean background for SDS-separated samples, the membrane must be pretreated for 5 minutes in 1% KOH. Afterwards, rinse the membrane in PBS and start the 'AuroDye forte staining procedure'.

In special cases, to obtain the highest sensitivity, the AuroDye forte signal can be silver-enhanced with the IntenSE BL silver enhancement kit (RPN492).

## 5.1. AuroDye forte staining procedure

1. The blot is incubated with excess PBS supplemented with 0.3% Tween-20 at 37°C for 30 minutes, and washed 3 times in PBS supplemented with 0.3% Tween-20 at room temperature for 5 minutes. Agitate during incubation and washing.
2. The washed blot is drained, washed briefly with distilled water and incubated in AuroDye forte. Sheets are incubated in sealed plastic bags and strips in stoppered tubes under constant agitation on a tilting apparatus. If a tilting apparatus is not available, best results are obtained by using excess AuroDye forte in a clear glass or polycarbonate tray on a reciprocal or rotary shaker. Incubation should continue until optimum colour formation has been obtained. In general, incubation time is in the order of 2 to 4 hours. Heavy protein bands require the longer time to become fully contrasted. Overstaining does not occur.
3. Wash the blot in distilled water and air dry. The stain does not fade.

## 5.2. Optional contrast enhancement procedure with IntenSE BL

In cases where an extremely high sensitivity or contrast is required, the AuroDye forte signal can be further amplified with IntenSE BL, the ready-to-use, light-insensitive silver enhancement system. Read the product information provided with the IntenSE BL kit carefully.

1. Handle blots by their edges with clean plastic tweezers. Stain the blot with AuroDye forte according to step 1 and 2 of the staining procedure explained above.
2. Wash the blot in distilled water for 3 x 5 minutes.
3. Pour equal volumes of enhancer and initiator solution together in a glass or plastic Petri dish and mix well. Immediately transfer the AuroDye forte stained blot into the silver enhancement mixture. User sufficient to completely submerge the membrane. Incubate under constant agitation for example on a rotary shaker.
4. Enhance the signal until a satisfactory enhancement is reached. The enhancement can be monitored and interrupted at will. Since there is usually a light background of colloidal gold, this will also be amplified. The researcher should determine the appropriate balance between signal enhancement and increased background.
5. Wash the membrane in several changes of excess distilled water for 15 minutes, and dry it between filter papers.

# 6. Applications

AuroDye forte is used for staining the proteins on duplicate blots of overlay assays to check the quality of the transfer, by comparing with a duplicate silver stained pattern in the gel and to make correlation of detected bands with the total protein transfer. For quick reference, blots may also include an additional lane with molecular weight makers. See our procedural insert of AuroProbe BLplus to more guidelines on obtaining high quality results with immunoblotting.

AuroDye forte may be used for high sensitivity staining of Western blots of 20-gels to maximize the number of stained spots. Optionally, the AuroDye forte signal can be further amplified with IntenSE BL.

AuroDye forte may be used to negatively stain transfers of nucleic acids on nylon-based membranes prior to hybridization. This facilitates correlation of hybridized bands with the total electrophoretogram.

# 7. Troubleshooting

**Observation: AuroDye forte turns purplish during staining.**

| Probable cause | Remedy |
|---|---|
| **1.** Agglutination of gold particles by proteins released from the blotting membranes. | **1.** Wash briefly in excess water. Replace AuroDye forte, if possible, use lower protein loads. This phenomenon is less frequently observed with PVDF-membranes because they retain proteins better than nitro cellulose. |

**Observation: AuroDye forte turns colourless during staining.**

| Probable cause | Remedy |
|---|---|
| **1.** Adsorption of all the gold particles by excess protein on blotting membrane. Impurities released from filter paper absorbed onto the blotting membrane during transfer. | **1.** Replace AuroDye forte and double the volume/$cm^2$. If possible, use lower protein loads. Use high quality filter paper in electro-transfer apparatus during blotting. |

**Observation: Spotty background.**

| Probable cause | Remedy |
|---|---|
| **1.** Impurities released from filter paper absorbed onto the blotting membrane during transfer. | **1.** Use high quality filter paper in electro-transfer apparatus during blotting. |

**Observation: High background.**

| Probable cause | Remedy |
|---|---|
| **1.1.** Interference by proteinaceous contaminants. | **1.1.** Use sandwich set-up as outlined on page 8. If possible, use lower protein loads. Replace Scotch-Brite and follow recommendations as outlined in the procedures on pages 8–10. |
| **1.2.** Optional silver enhancement time was too long. | **1.2.** Use a shorter silver enhancement time. |
| **1.3.** Interference by contaminants of chemicals. | **1.3.** Always use high quality chemicals. |

**Observation: Smears on background.**

| Probable cause | Remedy |
|---|---|
| **1.** Incorrect handling. | **1.** Handle blot with clean plastic tweezers and avoid contact with gloves. |

# 8. Staining procedure

Incubate in excess PBS with 0.3% Tween-20
30 minutes at 37°C

|

Wash extensively in excess PBS with 0.3% Tween-20
3 x 5 minutes at room temperature

|

Incubate in AuroDye forte
2 to 4 hours

|

Wash blot in excess distilled water for 15 minutes

Dry between filter papers

Optional silver enhancement
Wash blot in distilled water for 3 x 5 minutes.

|

Incubate with freshly prepared IntenSE BL silver enhancement mixture until a satisfactory contrast enhancement is obtained

|

Wash blot in excess distilled water for 15 minutes and dry between filter papers

# 9. Ordering information

**AuroProbe BLplus immunogold reagents**

| Product code | Product name | Biological agent |
|---|---|---|
| RPN460 | AuroProbe BLplus GAR | Goat anti-rabbit IgG(H+L) |
| RPN461 | AuroProbe BLplus GAM IgG | Goat anti-mouse IgG(Fc) |
| RPN462 | AuroProbe BLplus GAM IgM | Goat anti-mouse IgM(μ) |
| RPN463 | AuroProbe BLplus GAM IgG+1gM | Goat anti-mouse IgG+1gM(H+L) |
| RPN464 | AuroProbe BLplus GAHu | Goat anti-human IgG(H+L) |
| RPN465 | AuroProbe BLplus GARa | Goat anti-rat IgG(H+L) |
| RPN466 | AuroProbe BLplus RAG | Rabbit anti-goat IgG(H+L) |
| RPN467 | AuroProbe BLplus streptavidin | Streptavidin |

AuroProbe BLplus reagents are supplied as 2 ml units.

**IntenSE BL silver enhancement system**

| Product code | Product name | Unit |
|---|---|---|
| RPN492 | IntenSE BL kit | 500 ml |

Ready to use, light-insensitive silver enhancement reagent at neutral pH

**Additional reagents**

| Product code | Product name | Unit |
|---|---|---|
| RPN410 | Normal goat serum, lyophilized | Reconstitute in 5 ml |
| RPN411 | Normal rabbit serum, lyophilized | Reconstitute in 5 ml |
| RPN412 | BSA | 25 g |

**Hybond-ECL nitrocellulose membranes**

| Product code | Product name | Unit |
|---|---|---|
| RPN2020D | Hybond-ECL | 10 sheets<br>20 x 20 cm<br>membranes |
| RPN82D | Hybond-ECL | 50 sheets<br>82 mm diameter<br>discs |

# 10. Reference

**1.** SEGERS, J. and RABAEY, M. *Protides of the Biological Fluids*, **33**, p.589, 1985.

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