

AURION R-GENT SE-EM

High Efficiency Silver Enhancement Reagents
for
Electron Microscopy

PRODUCT INFORMATION

AURION R-GENT SE-EM INITIATOR, ACTIVATOR and ENHANCER constitute a Silver Enhancement Reagent which increases the average gold cluster or particle size by deposition of metallic silver facilitating observation at the electron microscopical level. The reagents are characterized by high efficiency and homogeneous silver deposition. The mixture is not sensitive to general light conditions.

AURION R-GENT SE-EM has been tailored for the enhancement of AURION GP-ULTRA SMALL reagents and is equally suited for the larger sized particles in the AURION EM reagents. The reagents are easy-to-use, the mixture has extremely delayed auto-nucleation and can be used under standard laboratory light conditions. The enhancement mixture has a pH value of 8.1-8.2.

AURION R-GENT SE-EM is available as a kit (500.033) containing 30 ml of ready-to-use ENHANCER, 3 ml of concentrated INITIATOR and 30 ml of ACTIVATOR. Additionally an empty 3 ml dropping bottle, labeled "DEVELOPER", is included. For pre-embedding silver enhancement a large volume kit is available (500.044) containing an additional 60 ml of ready-to-use ENHANCER.

AURION R-GENT SE-EM has a shelf life of 10 months when stored at 4°C. **Storage of the concentrated INITIATOR at -20°C is recommended.** AURION R-GENT SE-EM is allowed to reach room temperature before use.

Note: AURION R-GENT SE-EM is intended for research use only. Not for diagnostic or therapeutic use.

FIRST TIME USE

The ENHANCER solution is ready-to-use. The INITIATOR is a concentrated solution which must be diluted and activated before use, using the ACTIVATOR. This mixture compounds the DEVELOPER. An empty 3 ml dropping bottle is included to prepare the ready-to-use DEVELOPER.

Preparing the DEVELOPER.

1. Remove the dropper tip from the DEVELOPER bottle.
2. Remove the white cap from the ACTIVATOR bottle.
3. Holding the ACTIVATOR bottle upside down slowly give **40** drops into the DEVELOPER bottle. (see also ****Remark 1**)
4. Close the ACTIVATOR bottle
5. Remove the white cap from the INITIATOR bottle.
6. Holding the INITIATOR bottle upside down add **1** drop to the now partially filled DEVELOPER bottle. (see also ****Remark 1**)
7. Close the INITIATOR bottle.
8. Reinsert the dropper tip into the DEVELOPER bottle.
9. Close the DEVELOPER bottle and mix well on a vortex.

The now ready-to-use DEVELOPER has a shelf life of 1 month.

INSTRUCTIONS FOR USE

Silver enhancement speed is influenced by the temperature. For optimum reproducibility the temperature during enhancement should always be the same.

Silver enhancement is compatible with osmiumtetroxide fixed specimens. Application of osmiumtetroxide after the silver enhancement step might give improvement of ultra structure however. The use of Enhancement Conditioning Solution (500.055) is recommended when working with fragile specimens and is a prerequisite in double labeling protocols. Detailed information can be found in AURION NEWSLETTER NR. 5. Please keep in mind that using osmiumtetroxide after enhancement results in partial removal of the metallic silver.

ON-GRID ENHANCEMENT

The use of nickel grids is highly recommended, as nickel is relatively insensitive to silver enhancement. Gold or copper grids must not be used!

ACTUAL PROCEDURE

- 1 Allow the DEVELOPER and ENHANCER to reach room temperature.
- 2 After the immuno gold incubation step grids are washed and postfixed as described in the package inserts supplied with all AURION ImmunoGold reagents.
It is important to use several washing steps with distilled water in order to remove buffer components that may influence the enhancement. Grids are kept on drops of distilled water prior to enhancement.
- 3 **PREPARING THE ENHANCEMENT MIXTURE:**
Once temperature equilibrium has been established give **20** drops of the ENHANCER solution into a vial that will contain at least 1.5 ml, e.g. an Eppendorf vial. Make sure to keep the bottle upside down in a vertical position. Add **1** drop of the DEVELOPER solution, again making sure that the bottle is kept upside down in a vertical position. Mix well on a vortex. (see also ****Remark 2**)
- 4 **ENHANCEMENT PROCEDURE**
Post-embedding applications: Grids are floated on top of drops of the enhancement mixture on a sheet of parafilm. Transfer of the grids can be performed with fine non-magnetic tweezers. Preferably a metal loop is used which diminishes the risk of contamination and greatly facilitates transfer.
Enhancement time is typically between 20 and 30 minutes.

Pre-embedding applications: silver enhancement may be applied either before (1) or after OsO₄ fixation.
Ad (1): enhancement may have to be performed for a longer time period due to potential removal of silver by OsO₄. This effect is buffer related. When possible use OsO₄ in deionized water. Otherwise phosphate based buffers are recommended. Do not use OsO₄ in cacodylate or Tris buffers.
Alternatively enhancement may be done after ultra thin sectioning.

Enhancement is done at room temperature (preferably 20°C).

The composition of the enhancement mix prevents particles resulting from auto-nucleation to stick to specimens.
The actual enhancement time has to be established empirically and adjusted according to the desired particle growth.
- 5 When enhancement is complete the specimens are washed extensively with distilled water (at least 3x5 minutes). A postfixation with photographic fixer is not required.
- 6 After washing on-grid specimens may be contrasted according to standard procedures.
- 7 In order to preserve the silver signal it is recommended to store enhanced grids in a dry environment.

****Remark 1:** Uniformity of the diameter of the gold/silver particles using Aurion R-Gent SE-EM may be additionally improved by using a developer with an initiator:activator ratio of 1:60 (instead of the 1:40 ratio that is in general sufficient). This change in the composition of the developer will slow down silver enhancement and needs to be compensated by an increase in enhancement time of approximately 10 –15 minutes.

****Remark 2:** Enhancement rate is dependent on the concentration of Developer in the final mix. Reaction time is reduced by approximately 50% when using 2 drops of Developer in the final silver enhancement mix instead of 1 drop. This can be helpful in pre-embedding enhancement. Increasing the Developer concentration may have a negative effect on homogeneity of the gold/silver particles.

****Remark 3:** Depending on the characteristics of the specimen and silver enhancement time an overall yellow staining may occur. This does **not** interfere with the specific enhancement.

AUXILIARY REAGENTS

CODE	DESCRIPTION
500.055	ECS 10x concentrate, 100ml

Silver enhancement reagents for LM and bio-assays.

500.011	AURION R-GENT, 60ml
500.022	AURION R-GENT, 500ml



AURION

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