ENHANCEMENT CONDITIONING SOLUTION

PRODUCT INFORMATION

AURION Enhancement Conditioning Solution (ECS) substitutes the water washes prior to and after silver enhancement which may have a detoriating effect on ultrastructure. The use of AURION ECS is a prerequisite for successful pre-embedding double immunogold/silver labeling at the electron microscopic level (Yi et al., J. Histochem. Cytochem. 49(3), (2001), 279.

When using AURION ECS to protect the morphology of fragile specimens the silver enhancement time should be about 50% longer as compared to the time needed after fixation and water washes.

ECS has been tailored for combined use with AURION R-GENT SE-EM and is supplied as a 10x concentrate. Dilute with distilled water before use.

AURION ECS is stored at room temperature.

ACTUAL PROCEDURE (1)

Silver Enhancement of fragile specimens

1 Allow R-GENT SE-EM DEVELOPER and ENHANCER to reach room temperature.

2 After the immuno gold incubation step specimens are washed and postfixed as described in the package inserts supplied with all AURION ImmunoGold reagents.

3 Wash in Enhancement Conditioning Solution, 4 x 5 minutes. Washing is extended to 4 x 10 minutes for pre-embedding applications.

4 Prepare the enhancement mixture as described in the R-GENT SE-EM package insert.

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 30 and 45 minutes.

Pre-embedding applications: Specimens are silver enhanced in small vials or multi-well plates. Agitation is recommended. Enhancement may have to be performed for a longer time period (typically between 60 and 90 minutes) a.o. due to potential removal of silver by OsO4. Alternatively enhancement may be done after ultra thin sectioning.

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

The actual enhancement time has to be established empirically and adjusted according to the desired particle growth.

5 When enhancement is complete specimens are washed in Enhancement Conditioning Solution, 4 x 5 minutes. Washing is extended to 4 x 10 minutes for pre-embedding applications. A postfixation with photographic fixer is not required.

6 After washing on-grid specimens may be contrasted according to standard procedures. In order to preserve the silver signal it is recommended to store enhanced grids in a dry environment.

Pre-embedding specimens are washed in 0.1 M phosphate buffer for 2 x 10 minutes and post-fixed in 0.5% OsO4 in phosphate buffer for 15 minutes. After washing specimens are embedded in resin according to standard procedures.

Remark: 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.

ACTUAL PROCEDURE (2)

Pre-embedding double immunogold/silver labeling

1 Allow R-GENT SE-EM DEVELOPER and ENHANCER to reach room temperature.

2 After the mixed incubation with both primary antibodies (from different species) and the immunogold incubation step with the first Ultra-Small Gold conjugate specimens are washed in incubation buffer and PBS as described in the package inserts supplied with all AURION ImmunoGold reagents. Specimens are however not postfixed.

3 Wash in Enhancement Conditioning Solution, 4 x 10 minutes.

4 Prepare the enhancement mixture as described in the R-GENT SE-EM package insert. Specimens are silver enhanced in small vials or multi-well plates. Agitation is recommended. Suggested enhancement time is 90 minutes. The actual enhancement time has to be established empirically and adjusted according to the desired particle growth.

5 Enhancement is stopped in 0.03M sodium thiosulphate in ECS for 10 minutes.

6 Wash in Enhancement Conditioning Solution, 4 x 10 minutes.

7 Wash in incubation buffer and proceed with the incubation of the second Ultra-Small Gold conjugate. Specimens are washed and may be postfixed as described in the package inserts supplied with all AURION ImmunoGold reagents.

8 Wash in Enhancement Conditioning Solution, 4 x 10 minutes.

9 Prepare the enhancement mixture as described in the R-GENT SE-EM package insert. Specimens are silver enhanced in small vials or multi-well plates. Agitation is recommended. Suggested enhancement time is 60 minutes. The actual enhancement time has to be established empirically and adjusted according to the desired particle growth.

10 Wash in Enhancement Conditioning Solution, 4 x 10 minutes.

11 Specimens are washed in 0.1 M phosphate buffer for 2 x 10 minutes and post-fixed in 0.5% OsO_4 in phosphate buffer for 15 minutes. After washing specimens are embedded in resin according to standard procedures.

Remark: 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 PRODUCTS

CODE	DESCRIPTION
500.033	AURION R-GENT SE-EM, 30ml
500.044	AURION R-GENT SE-EM, 90ml

AURION
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