#### PRODUCT INFORMATION

AURION R-GENT SE-INTENSE DEVELOPER 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 microscopical level. The generated black signal is easily detected in bio assays and is compatible with counterstaining in light microscopy.

AURION R-GENT SE-INTENSE has been tailored for the enhancement of AURION GP-ULTRA SMALL reagents and AURION 2nm ImmunoGold Reagents, is however equally suited for the larger sized particles in the AURION Conventional ImmunoGold reagents.

The silver enhancement reagent is easy-to-use, the mixture has extremely delayed auto-nucleation and can be used under standard laboratory light conditions.

AURION R-GENT SE-INTENSE is available as a kit in two unit sizes (2x30 ml or 2x250 ml) and consists of a separate DEVELOPER and ENHANCER. The components are mixed in equal amounts immediately before use.

IMPORTANT: do not exchange the screw caps: the <u>green</u> cap should be used for the DEVELOPER flask, the <u>white</u> cap should be used for the ENHANCER flask. For ease of recognition the DEVELOPER flask has been labeled with a <u>green</u> dot.

AURION R-GENT SE-INTENSE is stored at 4°C and allowed to reach room temperature before use.

#### INSTRUCTIONS FOR USE

Silver enhancement speed is influenced by the temperature. For optimum reproducibility the temperature during enhancement should always be the same. This is especially important for microscopy applications.

Standard enhancement temperature is 20°C. Enhancement may be speeded up by increasing the temperature to 26°C.

## BIO ASSAYS AND LIGHT MICROSCOPY

### ENHANCEMENT SET UP

Microscopical slides can be incubated with small amounts of the freshly prepared enhancement mixture (100 - 200  $\mu$ l) in a moist chamber.

Cover slips may be enhanced in a six-well culture plate as an alternative.

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

# ACTUAL PROCEDURE

- 1 Allow the DEVELOPER and ENHANCER to reach room temperature.
- 2 After the immuno gold incubation step specimens are washed (and postfixed in glutaraldehyde) 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. Specimens are kept in deionized water prior to enhancement.

- 3 Once the DEVELOPER and ENHANCER reagents have reached temperature equilibrium, equal parts are mixed immediately before applying the enhancement mixture to the specimens. The specimens should be fully covered by the mixture.
- 4 Enhancement is done at room temperature (preferably 20°C). Gentle shaking is recommended.
- 5 Typical enhancement times are between 15 and 25 minutes. Auto-nucleation is visible only after 35-45 minutes. The on-going process may be monitored using an inverted light microscope with dimmed light conditions. In this way heat transfer to the enhancement mixture is kept low.
- 6 When enhancement is complete (judged by the presence of the black signal developed) the specimens are washed extensively with distilled water (at least 3x5 minutes). A postfixation with photographic fixer is not required.
- 7 After washing light microscopical specimens may be counterstained according to standard procedures.
- 8 In order to preserve the silver signal and to prevent fading it is necessary to dehydrate the silver enhanced specimens and to mount in water-incompatible media such as Pertex (HistoLab, Sweden).

#### ELECTRON MICROSCOPY

For electron microscopy applications we strongly recommend AURION R-GENT SE-EM (see auxiliary products), a highly efficient and light insensitive silver enhancement system with **low** viscosity.

Silver enhancement is compatible with osmiumtetroxide fixed specimens. Using osmiumtetroxide fixation after enhancement results in partial removal of the metallic silver.

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

AUXILIARY PRODUCTS

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

