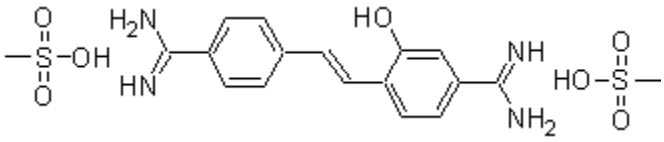
Hydroxystilbamidine

Ordering Information	*Storage Conditions*
Product Numbers: 17514	Avoid exposure to light Keep < -15 °C and desiccated Expiration: 12 months upon receipt

Chemical and Physical Properties



Molecular Weight: 472.54
Appearance: Yellow powder
Solvents: Water or 0.9% PBS
Spectral Properties: Excitation = 360 nm; Emission = 625 nm.

Biological Applications

Hydroxystilbamidine (also called Fluoro Gold) is used for staining DNA and RNA. It exhibits distinctively different fluorescence emission profiles when bound to DNA compared to RNA. This cationic dye is also frequently used as a retrograde neuronal tracer.

Sample Protocol for Histological Staining

The use of Hydroxystilbamidine is essentially the same as other fluorescent tracers. The main difference is that Fluoro-Gold is more flexible in terms of post-injection survival times, concentration range, tissue treatment and compatibility with other histochemical techniques. It is also more resistant to fading, brighter and more permanent than most other fluorescent tracers.

1. **Dye Concentrtation:** 1to 10% Hydroxystilbamidine has been successfully used. Initially, a 4% concentration is advised. If undesirable necrosis occurs at the injection site, or labeling is too intense, reduce the concentration to a 2% solution.
2. **Dye Administration:**
   2.1 Pressure injection - this is probably the most frequently used mode of application. Volumes injected range from 0.05-1 µl, typically 0.1-0.2 µl.
   2.2 Crystal - a crystal of the tracer can be administered from the tip of a micro-pipette.
3. **Fixation:** Although any fixative, or no fixative, can be used, PBS containing 4% formaldehyde is most frequently employed. Fixatives containing high concentrations of heavy metals (eg. osmium, mercury) will quench the fluorescence, while high concentrations (over 1%) of glutaraldehyde may increase background fluorescence.
4. **Histochemical Processing:** Tissue containing Hydroxystilbamidine may be processed according to virtually any common histological technique. Frozen sections of fixed tissue are most frequently used.
5. **Combined Methods:** At this point of processing, sections may be further processed for a second marker such as autoradiography, HRP histochemistry, immunocytochemistry, a second fluorescent tracer, fluorescent counterstain, etc.
6. **Mounting, Clearing and Coverslipping:** Sections are typically mounted on gelatin-coated slides, air-dried, immersed in xylene, and coverslipped with nonfluorescent DPX plastic mounting media. Sections may be dehydrated with graded alcohols, unless this is not compatible with a second tracer. If

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Hydroxystilbamidine is to be combined with fluorescence immunocytochemistsry, then sections are air-dried and directly coverslipped with DPX.

7. **Examination and Photography:** Hydroxystilbamidine can be visualized with a fluorescence microscope using a wide band ultraviolet excitation filter (excitation - 323 nm, emission - 620 nm at neutral pH). A gold color is emitted when tissue has been processed with neutral pH buffer, whereas a blue color is emitted when tissue is processed with acidic (eg. PH 3.3) pH buffer. It can be photographed digitally or with film (use Ektachrome 200-400 ASA film for color prints and comparable speed film for black and white prints). Most exposure times range from 10-60 second exposures, depending on the objective magnification and the intensity of the label. Thirty (30) second exposures are about average. Multiple exposures may be exploited to simultaneously visualize Hydroxystilbamidine and another tracer. Thus, UV would be combined with bright field illumination to simultaneously locate Fluoro-Gold with HRP or silver grains in autoradiography. Similarly, blue light excitation can be combined to also visualize the green emission color of FITC, while green excitation light may be used to simultaneously observe the red emission color of propidium iodide, or ethidium bromide (a fluorescent counterstain).

References

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