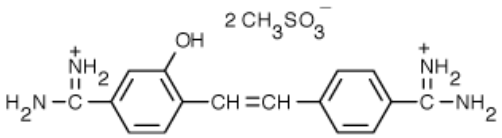
FT-40766A

Hydroxystilbamidine, methanesulfonate

Product Information

Name :	**Hydroxystilbamidine methanesulfonate**
	also know as **FluoroGold®**
Catalog Number :	FP-40766A, 10mg
	FP-JW7290, 200 µl (4% solution)
Structure :	$C_{18}H_{24}N_4O_7S_2$
Molecular Weight :	MW= 472.53
Soluble:	H2O or DMSO
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (pH4.5) = 323 / 408 nm; EC: 33 000 M^{-1} cm^{-1} $\lambda_{exc}\backslash\lambda_{em}$ (membrane bound) = 350-395 / 530-600 nm $\lambda_{exc}\backslash\lambda_{em}$ (DNA) = 360 / ~450,~625; EC: 27 000 M^{-1} cm^{-1}



Hydroxystilbamidine is also available in bulk (UP544200) and in solution at 4% (FP-JW7290)

Storage: +4°C (-20 °C for long term) and protect from light, desiccated (K)

Introduction

Hydroxystilbamidine has been used extensively as a retrograde tracer for neurons and also a histochemical stain. Compared with other fluorescent tracers, the main difference is that Hydroxystilbamidine is more flexible in terms of post-injection survival times, concentration range, tissue treatment and compatability.

Advantages of Hydroxystilbamidine when used alone:

- Intensively vibrant retrograde fluorescent tracer that is extremely sensitive and reliable
- Not taken up by undamaged fibbers of passage
- Does not diffuse out of retrogradely labeled neurons
- Wide range of survival times from one day to over a month
- Stable fluorochrome that takes superlative colour and black and white photos
- Easy and safe – long shelf life- pure reliable source
- Can be pressure injected or iontophoresed
- Compatible with most other frequently used neuroanatomical techniques *

*such as immunofluorescence, immunocytochemistry, autoradiography,
HRP histochemistry, paraffin embeded and other retrograde flurescent tracers

Avantages of Hydroxystilbamidine when enhanced by the antibody:

- Substantially enhances visualization of Hydroxystilbamidine
- Allows much more precise anatomical mapping
- Shows extremely fine anatomical structure including dendrites axons and terminals
- Permanent staining when used with enzyme amplification kits

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Directions for use

1. STORAGE AND SELF-LIFE

Dry Hydroxystilbamidine should be kept in the dark at –4°C, or –20°C for long term storage, but is also stable at room temperature for well over six months. The dye in solution should be kept in the dark at 4 degrees celcius and will remain stable for at least six months.

2. DYE ADMINISTRATION

Hydroxystilbamidine can be dissolved in distilled water or 0.9% saline, or utilized as a suspension in 0.2M neutral phosphate buffer.

Hydroxystilbamidine has been successfully used at concentrations ranging from 1-10%. 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.

A. Pressure Injection: this is probably the most frequently used mode of application. Volumes injected range from .05-1 ul, typically .1-.2 ul.

B. Iontophoresis: discrete, small injection sites result from 4-10 second pulsed iontophoretic (+5 to +10ua/10min) application.

C. Crystal: a crystal of the tracer can be administered from the tip of a micro-pipette.

3. POST-OPERATIVE SURVIVAL PERIOD

Good retrograde labeling has been observed with periods ranging from two days to two months. Survival periods of three to five days are typical. Long survival periods enhance filling of distal processes without diffusion of the dye from the cell.

4 FIXATION

Most any fixative, or no fixative, can be used, Phosphate neutral buffered saline containing 4% formaldehyde is frequently employed. Fixatives containing high concentrations of heavy metals (e.g. osmium, mercury) will quench the fluorescence, while high concentrations (over 1%) of glutaraldehyde may increase background fluorescence

5 HISTOCHEMICAL PROCESSING

Tissue containing Hydroxystilbamidine may be processed according to virtually any common histological technique. This includes cryostat sections of unfixed tissue (10 µm), frozen sections of fixed tissue (20 µm), and thin sections cut from tissue imbedded in either plastic (2-4 µm) or paraffin (3-10 µm). Frozen sections of fixed tissue are most frequently used.

6 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.

7 MOUNTING, CLEARING, COVERSLIPPING

Sections are typically mounted on gelatin-coated slides, air-dried, immersed in xylene, and coverslipped with non-fluorescent DPX plastic mounting media. Sections may be dehydrated with graded alcohols, unless this is not compatible with a second tracer. If Hydroxystilbamidine is to be combined with fluorescence immunocytochemistry, then sections are air-dried and directly coverslipped with neutral buffered glycerine (1:2).

8 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 (e.g. 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, for example Tri-X). 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 Hydroxystilbamidine with HRP or silver grains in autoradiography. Similarly, blue light

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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).

Additional Information

VEHICLE

For pressure injections through a microsyringe or micropipette, Hydroxystilbamidine should be dissolved in distilled water or .9% saline. Hydroxystilbamidine may also be utilized as a suspension in .2M neutral phosphate buffer, however, the suspended particles may clog a fine micropipette tip so distilled water or .9% saline is the preferred vehicle. For iontophoresis, a 1% Hydroxystilbamidine solution is made up in .1M acetate buffer (pH=3.3). Well-cleaned (95% ETOH, water) glass micropipettes should have tips of 10-20 um. Optimal iontophoresis parameters are +1 to +5u amps delivered with pulsed current (4-10 seconds on, 4-10 seconds off) over a 10-20 minute period.

INJECTION SITES

Virtually any central or peripheral nervous system structure can be injected with Hydroxystilbamidine for analysis of retrograde transport. In the peripheral nervous system, ganglia and peripheral targets can be studied. For studies of peripheral nerve, the nerve should be cut or damaged and either dipped in, or injected with, aq 5% solution of Hydroxystilbamidine. Since Hydroxystilbamidine is not significantly taken up by intact fibers of passage, the fibers must be cut or severely damaged for uptake of the dye to occur. It is not presently known if Hydroxystilbamidine can be used for intracellular injections or used to study gap junctions, however, some groups of investigators are presently testing these possibilities.

TRANSPORT AND SURVIVAL TIME

Hydroxystilbamidine is used as a retrograde axonal tracer, although orthograde axonal transport does occur. The survival time should be varied (especially to very short survival times of 12 hours - 2 days) to maximize orthograde transport in the specific neuronal system under study. For retrograde transport, the survival times should be varied from 4 days to 14 days. Seven to 10 days works for most systems, although long pathways (e.g., spinal cord to brainstem) and pathways in large mammals (e.g., cats, monkeys) may require longer survival times (e.g., 14 days). In addition, since Hydroxystilbamidine remains fast within retrogradely labeled neurons, survival times of several months will also produce
excellent results. For iontophoresis, 1 2-5 day survival time is recommended.

TISSUE PROCESSING

Tissue processing is covered in detail in the use guide and in the original publication (Schmued and Fallon, 1986, Brain Research 377:147-154). Since Hydroxystilbamidine is stable in many solvents and remains fast within retrogradely labeled neurons, it's use is compatible with many histochemical techniques. It can be used with other retrograde tracers, immunofluorescence, PAP and ABC immunocytochemistry, HRP histochemistry, autoradiography, counterstains (ethidium bromide is the preferred fluorescent counterstain), paraffin embedding and plastic embedding. However, if tissue is unfixed, additional processing of tissue in aqueous solutions for over an hour or two will result in loss of Hydroxystilbamidine fluorescence from labeled neurons. Hydroxystilbamidine may be useful in electron microscopy. Hydroxystilbamidine can be used in a brain which has been sectioned and transferred to phosphate buffer. Sections are typically mounted on gelatin-coated slides, air dried, immersed in xylene and coverslipped with DPX plastic mounting media (FLUKA Chemical Corp., 255 Oser Avenue, Hauppauge, New York, 11788, Catalog #44581). Tissue may also be viewed on slides without further processing, can be run through graded alcohols for dehydration, or, for immunocytochemistry, the sections can be air dried and directly coverslipped with neutral buffered glycerine (1:2).

EXAMINATION AND PHOTOGRAPHY

Hydroxystilbamidine is visualized with a fluorescence microscope using a wide band ultraviolet (UV) excitation filter. Use the same filter pack you would for other fluorescent retrograde tracers excited under wide band UV (e.g., True Blue, Fast Blue, Nuclear Yellow), such as the Leitz Ploem filter system A (Wide Band UV, Excitation filter BP 340-380), Mirror RKP 400, Barrier Filter LP 430). Objectives should be made especially for fluorescence microscopy (such as that made by Zeiss) glycerine, or water. Since plastic does absorb UV light, it is not advised to view through plastic petri dishes, etc. Recommended films are T-Max (Kodak, black & white) and Ektachrome 200 (Kodak, color slides). Exposure times usually vary from 20 seconds to 1.5 minutes.
It is noteworthy that a photobleaching of Fluorogold reported initially due to the immunohistochemical processing, is in fact due to the water in the sections. This problem is easily corrected by using a 29:1 or 19:1 glycerine-PBS buffer mounting medium.

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PHYSICO-CHEMICAL CHARACTERISTICS

Fluorogold is provided as golden-yellow crystalline powder, with highest purity. Lower purity compounds have been shown to have very different properties. It can be solubilized easily at 5% w/v in aqueous solutions. A 1% solution has a pH between 4.0 and 5.2 at 25°C.

The spectral characteristics vary with pH. It has a wide emission bandwidth in water, $\lambda_{abs./}\lambda_{em.}$ [2%, pH4.5] =323/408nm; EC: 33 000 M^{-1} cm^{-1}, with a second peak at ~600 nm. Spectra differ greatly when bound to membranes at a physiological pH of 7.4 ($\lambda_{exc}\backslash\lambda_{em}$= 350-395 / 530-600 nm), to DNA ($\lambda_{exc}\backslash\lambda_{em}$ = 360 / ~450,~625nm; EC: 27 000 M^{-1} cm^{-1}), or to RNA.
FluoroGold exhibits AT-selective binding/II structure. Complex fluorescent properties allow DNA/RNA distinction.

References

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- **Cameron A**. *et al*., Genetic Manipulation of Intraspinal Plasticity after Spinal Cord Injury Alters the Severity of Autonomic Dysreflexia, *The Journal of Neuroscience,* 26(11):2923-2932 (2006) Article
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- **Xu P**. *et al.,* Activin Induces Tactile Allodynia and Increases Calcitonin Gene-Related Peptide after Peripheral Inflammation, *The Journal of Neuroscience*, 25(40):9227-9235 (2005) Article

Related products

- Fast Blue, FP-CM0240
- True Blue Chloride, FP-M1159A
- Nuclear Yellow, FP-M1477B
- Anti Hydroxystilbamidine Polyclonal Rabbit Antibody, Unconjugated, CK2901
- Histochoice Mounting media, 41927A
- Ethidium bromide, FP-06022A
- Propidium iodide, FP-31238B, FP-36774A

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Fluoprobes / Interchim; Hotline : +33(0)4 70 03 73 06

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