Revised: February 12, 2014

Product Information

PathoGreen™ Histofluorescent Stain, 1000X

Catalog Numbers: 80027-5mL and 80027-50mL

Storage and Handling: Product ships at room temperature. Upon receipt, store at -20°C and protect from light. Product is stable for at least one year from date of receipt when stored as directed.

Spectral Properties: Ex/Em maxima: 497/520 nm

Product Description

PathoGreen™ Histofluorescent Stain is an anionic green fluorescent dye functionally similar to Fluoro-Jade® dyes. These dyes stain degenerating neurons and their processes after exposure to a variety of neurotoxic insults in brain sections and cultured neurons. The mechanism of neuronal staining by anionic fluorescent dyes has not been determined. It has been proposed that the negatively charged dyes bind to positively charged polyamines or other molecules specifically generated in dying neurons.

Reference**:** Schmued, L.C. and Hopkins, K.J. Fluoro-Jade: Novel Fluorochromes for Detecting Toxicant-Induced Neuronal Degeneration. Toxicol Pathol 28, 91 (2000).

Staining protocol for tissue sections

1. Mount vibratome sections on gelatin coated slides and dry on a slide warmer at 50-60°C for at least 30 minutes. For frozen sections, warm slides to room temperature. For paraffin sections, deparaffinize, rehydrate to water, and proceed to step 5.
2. Fix sections in basic alcohol (20 mL 1% NaOH (w/v in water) + 80 mL absolute ethanol) for 5 minutes at room temperature.
3. Incubate slides in 70% ethanol for 2 minutes.
4. Incubate slides in dH_2O for 2 minutes.
5. Incubate slides in 0.06% potassium permanganate in dH_2O for 10 minutes.

   Note: potassium permanganate reduces background fluorescence, but may alter protein antigens in tissue sections; the potassium permanganate incubation time may need to be reduced for combined PathoGreen/immunofluorescence staining.
6. Rinse slides twice with dH_2O, and incubate in dH_2O for 2 minutes.
7. Prepare 1X PathoGreen™ staining solution by diluting 1000X PathoGreen™ stock solution 1:1000 in 0.1% acetic acid in dH_2O.

   Note: Use 1X PathoGreen™ staining solution within one day.

   Optional: for blue fluorescent nuclear counterstaining, add DAPI (catalog number 40043) to 1X PathoGreen™ staining solution at a final concentration of 1 ug/mL.
8. Incubate slides in 1X PathoGreen™ staining solution for 10 minutes.
9. Rinse slides 3 X 1 minute in dH_2O.
10. Air dry slides on a slide warmer at 50-60°C for at least 5 minutes.
11. Incubate slides in xylene for 1-5 minutes.

    Note: do not perform alcohol dehydration prior to the xylene rinse, because this will extract the stain.
12. Coverslip slides with DPX mounting medium.

    Note: PathoGreen is not compatible with aqueous mounting medium, which will extract the stain.
13. Image fluorescence using a fluorescein filter set.

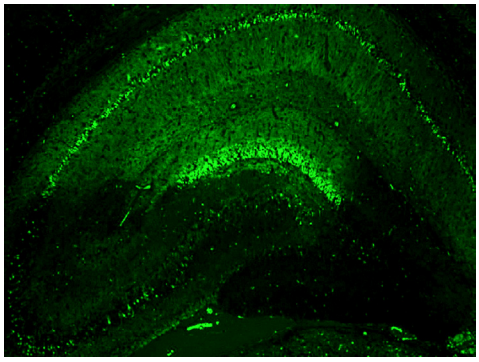

Figure 1. Degenerating neurons in a section of mouse hippocampus stained with PathoGreen™ Histofluorescent Stain.

Related Products

Cat.#	Product Name	Unit Size
40043	DAPI, 10 mg/mL in H_2O	1 mL
80014	Hydroxystilbamidine (equivalent to Fluoro-Gold™)	10 mg
80023	Hydroxystilbamidine (equivalent to Fluoro-Gold™), 4% in H_2O	200 uL
90057	Biotin ethylenediamine, hydrobromide (equivalent to Neurobiotin™)	25 mg
80026	Lucifer Yellow CH, lithium salt, 100 mM in H_2O	100 uL
92152	CF™488A hydrazide	1 mg
92154	CF™568 hydrazide	1 mg
92158	CF™594 hydrazide	1 mg
92136	CF™647 hydrazide	1 mg
70020	SynaptoGreen™ C4 (equivalent to FM®1-43)	5 mg
70021	SynaptoRed™ C2 (equvalent to FM®4-64)	5 mg
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses	1 pen
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen

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