USER GUIDE

Methyl Green

Together we breakthrough™

Cat. No. H-3402

Storage 15–25 °C

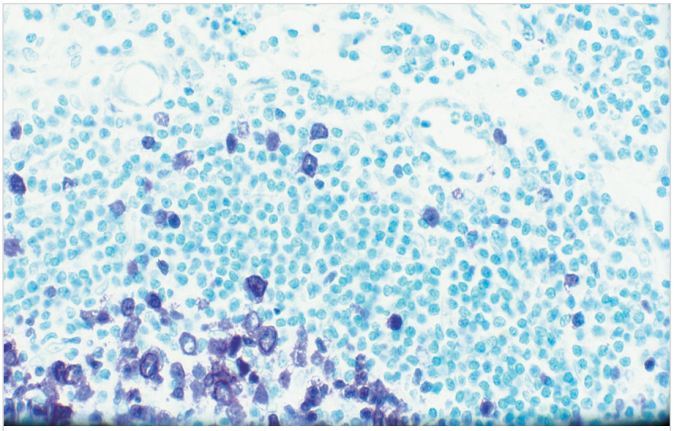
Description

This ready-to-use nuclear counterstain is designed to be used after completion of immunohistochemical staining or for routine histology. Nuclei in stained sections will be light green.

Counterstain incubation times should be optimized for each tissue type, antigen unmasking protocol, and nuclear staining intensity desired.

Components

Product Name	Volume
Methyl Green	500 ml



Tonsil section showing specific cell staining with Vector® VIP Substrate (purple). Counterstained with Methyl Green (green).

Instructions for Use

1. Rinse slides in tap water.
2. Staining sections with Methyl Green counterstain can be accomplished in two ways:

a. For individual slides, apply room temperature Methyl Green solution to sections. Incubate slides at 60 °C on a slide warmer or on a metal plate in an oven for 1–5 minutes. If using high temperature antigen unmasking techniques, longer incubation times (of up to 5 minutes) in methyl green solution will be required. After incubation, rinse slides with deionized water until rinse water is clear.

b. For batch processing, preheat Methyl Green solution to 60 °C in a Coplin jar or staining dish. Add slides and incubate at 60 °C for 1–5 minutes. If using high temperature antigen unmasking techniques, longer incubation times (of up to 5 minutes) in methyl green solution will be required. After incubation, remove slides and rinse with deionized water until rinse water is clear.†

3. Wash slides for 1 minute in deionized water. Remove slides and tap to eliminate excess water.
4. Dip slides 5–10 times in acetone containing 0.05% (v/v) acetic acid.††
5. Immediately dehydrate through 95% and 100% ethanol, clear, and permanently mount slides. Methyl Green is not compatible with aqueous mountants.

Notes

- See reverse side for substrate compatibility.
- Use ordinary precautions to avoid contact with skin and eyes.
- This product is for research use only.

† Methyl green solution may be reheated and reused.

†† This differentiation step can be reduced or eliminated if non-nuclear staining is not a problem. Omission of step 4 may result in a more intense nuclear stain.

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Counterstain/Substrate Compatibility Table

This table is designed as a reference to determine the optimal counterstain/substrate combination for your application. Considerations should be given to tissue type, antigen unmasking protocol and other detection parameters to achieve the desired staining intensity.

Substrate	Catalog Number	Hematoxylin and Hematoxylin QS H-3401 and H-3404	Methyl Green H-3402	Nuclear Fast Red H-3403
ImmPACT® DAB (brown)	SK-4105	Excellent Contrast	Excellent Contrast	Fair Contrast
ImmPACT DAB EqV	SK-4103	Excellent Contrast	Excellent Contrast	Fair Contrast
DAB (brown)	SK-4100	Excellent Contrast	Excellent Contrast	Fair Contrast
DAB-Ni (gray-black)	SK-4100	Excellent Contrast	Fair Contrast *	Good Contrast
ImmPACT AEC (red)	SK-4205	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
ImmPACT AMEC Red (red)	SK-4285	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
AEC (red)	SK-4200	Excellent Contrast	Counterstain Incompatibility **	Color Incompatibility
TMB (blue)	SK-4400	Color Incompatibility	Counterstain Incompatibility	Excellent Contrast
ImmPACT VIP (purple)	SK-4605	Fair Contrast	Excellent Contrast	Poor Contrast
Vector VIP (purple)	SK-4600	Fair Contrast	Excellent Contrast	Poor Contrast
ImmPACT SG (blue-gray)	SK-4705	Poor Contrast	Good Contrast	Excellent Contrast
SG (blue-gray)	SK-4700	Poor Contrast	Good Contrast	Excellent Contrast
ImmPACT NovaRED® (red)	SK-4805	Excellent Contrast	Excellent Contrast ***	Color Incompatibility
Vector NovaRED (red)	SK-4800	Excellent Contrast	Excellent Contrast ***	Color Incompatibility
ImmPACT Vector Red (magenta)	SK-5105	Excellent Contrast	Excellent Contrast	Color Incompatibility
Vector Red (magenta)	SK-5100	Excellent Contrast	Excellent Contrast	Color Incompatibility
Vector Black (black)	SK-5200	Excellent Contrast	Excellent Contrast *	Excellent Contrast
Vector Blue (blue)	SK-5300	Color Incompatibility	Good Contrast	Excellent Contrast
BCIP/NBT (indigo)	SK-5400	Color Incompatibility	Excellent Contrast *	Excellent Contrast

* This substrate shows a slight decrease in sensitivity following the methyl green protocol. This decrease can be minimized by reducing the heat incubation and acetone rinse times in the methyl green protocol.

** Substrate dissolves in acetone wash.

*** A slight color change in ImmPACT NovaRED and Vector NovaRED reaction product may be seen using methyl green.

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