USER GUIDE

VECTASHIELD® HardSet™

Antifade Mounting Medium with DAPI

Together we breakthrough™

Cat. No.	**H-1500**
Storage	Refrigerate
Concentration of DAPI	1.5 µg/ml
Refractive Index	1.36 (initial) 1.46 (cured)

Description

VECTASHIELD HardSet Antifade Mounting Medium preserves fluorescence and hardens after coverslipping. VECTASHIELD HardSet Antifade Mounting Medium's unique, stable formula prevents rapid photobleaching of fluorescent proteins and fluorescent dyes (see website for compatibility).

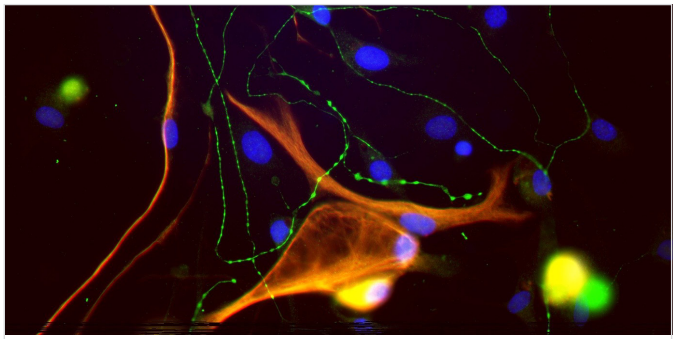
Each lot is tested for photobleaching retardance with qualitative and quantitative methods.

This formulation of VECTASHIELD HardSet Antifade Mounting Medium contains 4',6-diamidino-2-phenylindole (DAPI), which fluoresces when bound to DNA and is used as a nuclear or chromosomal counterstain. DAPI excites at about 360 nm and emits at about 460 nm when bound to DNA, producing a blue fluorescence, and is ideal as a nuclear or chromosomal counterstain.

Application

To mount tissues or cells on a slide dispense VECTASHIELD Mounting Medium onto the specimen using a disposable pipet or pipet tip. Small drop volumes of approximately 25 µl (per 22 mm x 22 mm coverslip) are recommended. Coverslip the specimen and allow VECTASHIELD Mounting Medium to disperse over the entire section. Tightly seal the bottle with the screw cap after use and store in an upright position.

After approximately 15 minutes at room temperature, the coverslip will become immobilized, and optimal antifade ability and refractive index will be achieved. After curing at room temperature for 15 minutes, slides can be placed at 4 °C, and the mounting media will harden completely overnight. Mounted slides should be stored at 4 °C or -20 °C, protected from light. For prolonged storage, -20 °C is recommended. If retraction occurs during prolonged storage, remove coverslip and remount. Coverslips can be easily removed by soaking in PBS overnight.



Dorsal root ganglia cells (neurons and satellite glia) and astrocytes stained for BetaIII tubulin (mouse) followed by DyLight™ 488 Anti-Mouse IgG (green), and GFAP (rabbit) followed by DyLight 549 Anti-Rabbit IgG (orange). Mounted in VECTASHIELD HardSet Antifade Mounting Medium with DAPI (blue).

Notes

When mounting thick or larger sections or larger coverslips, adjustments to the mounting protocol may be required. Increase volume of mountant to provide even coverage over entire specimen extending to all edges of the coverslip. Use of non-hardening VECTASHIELD Antifade Mounting Medium (H-1200) may be preferred.

The nuclear counterstains, DAPI and PI, will diffuse out of nuclei over time if the cells have been fixed in 100% methanol. In this case non-hardening VECTASHIELD Antifade Mounting Medium may be preferred.

DAPI is a mutagen and appropriate precautions should be taken when using it.

VECTASHIELD Antifade Mounting Media Products:

Product	Counterstain	Cat. No.	Unit Size	Hardening
VECTASHIELD® Antifade Mounting Medium	none	H-1000	10 ml	no
	DAPI	H-1200	10 ml	no
	PI	H-1300	10 ml	no
VECTASHIELD® PLUS Antifade Mounting Medium	none	H-1900	2 ml, 10 ml	no
	DAPI	H-2000	2 ml, 10 ml	no
VECTASHIELD® HardSet Antifade Mounting Medium	none	H-1400	10 ml	yes
	DAPI	H-1500	10 ml	yes
	TRITC-Phalloidin	H-1600	10 ml	yes
VECTASHIELD Vibrance® Antifade Mounting Medium	none	H-1700	2 ml, 10 ml	yes
	DAPI	H-1800	2 ml, 10 ml	yes

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