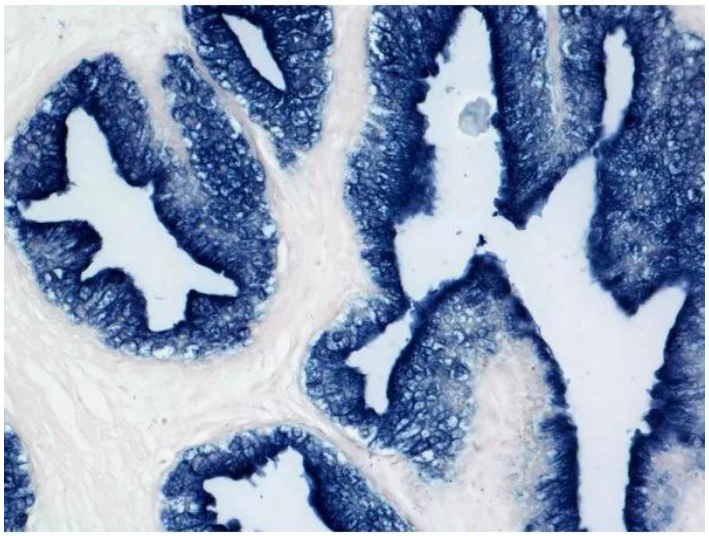
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BCIP/NBT SUBSTRATE KIT, ALKALINE PHOSPHATASE (AP), (5-BROMO-4-CHLORO-3-INDOLYL PHOSPHATE/NITROBLUE TETRAZOLIUM)

SKU: SK-5400



DESCRIPTION

BCIP/NBT AP Substrate Kit forms an indigo, heat stable reaction product. This chromogen can be used singly or in combination with other alkaline phosphatase or peroxidase substrates for multiple label applications.

Features:

- Greater sensitivity than conventional substrates
- Consistent and reliable
- Ideal for IHC/ICC/ISH and blots
- Fluorescent
- Heat Stable
- Permanent mounting

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• No wait times for mixing and dissolving powders or tablets
• Sufficient reagents to produce 200 ml of working solution
• Excitation: 645-685 nm*
• Emission: peaks at 823 and 855 nm**Biotechniques (2007) 42:756-759 and BioTechniques (2014) 57:254-256

SPECIFICATIONS

Microscopy	Brightfield, Fluorescence, Spectral Imaging
Unit Size	1 Kit
Applications	Immunohistochemistry / Immunocytochemistry, Immunofluorescence, In situ hybridization, Blotting Applications, Elispot
Additional Substrate Properties	Contrast with Tissue Pigments, Fluorescent, Heat Resistant, Multiple Labeling

KIT COMPONENTS

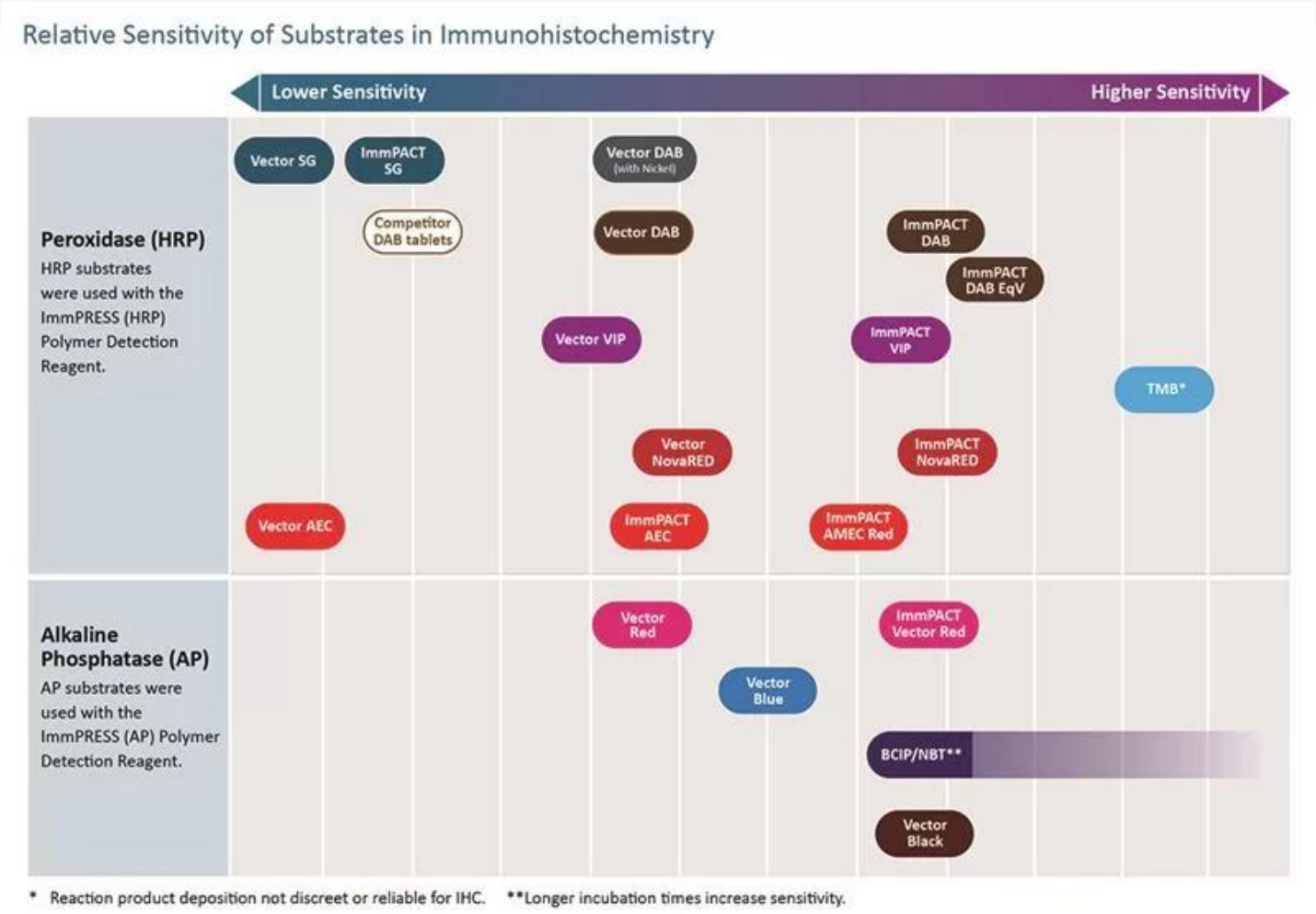
- 4 ml BCIP/NBT Reagent 1
- 3 ml BCIP/NBT Reagent 2
- 4 ml BCIP/NBT Reagent 3

TECHNICAL INFORMATION

This substrate can be used for developing blots or for in situ hybridization. At 20-30 minute development times, the sensitivity of this substrate is equivalent to the other alkaline phosphatase substrates. However, BCIP/NBT will continue to develop from several hours to overnight making it one of the most sensitive chromogenic substrates. This kit contains stock solutions in convenient dropper bottles.

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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	Vector Hematoxylin and Hematoxylin QS H-3401 and H-3404	Vector Methyl Green H-3402	Vector 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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Enzyme Substrate Combinations

Recommended combinations of substrates and the recommended order in which they should be used.

First Substrate \ Second Substrate	Alkaline Phosphatase			Peroxidase					
	ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100	Vector Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	ImmPACT VIP & Vector VIP (purple) SK-4605 & SK-4600	ImmPACT DAB, ImmPACT DAB EqV & DAB (brown) SK-4105, SK-4103, SK-4100	DAB-Ni (gray-black) SK-4100	ImmPACT NovaRED & Vector NovaRED (red) SK-4805 & SK-4800	ImmPACT SG & SG (blue-gray) SK-4705 & SK-4700	ImmPACT AEC, ImmPACT AMEC Red & AEC (red) SK-4205, SK-4285, SK-4200
Alkaline Phosphatase									
ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100		−	−	−	+	+	−	+	−
Vector Blue (blue) SK-5300	+		−	+	+	+	+	+	+
BCIP/NBT (indigo) SK-5400	+	−		+	+	+	+	+	+
Peroxidase									
ImmPACT VIP & Vector VIP (purple) SK-4605, SK-4600	−	+	−		+	+	−	+	−
ImmPACT DAB, ImmPACT DAB EqV & DAB (brown) SK-4105, SK-4103, SK-4100	+	+	+	+		−	−	+	+
DAB-Ni (gray-black) SK-4100	+	−	−	+	+		+	−	−
ImmPACT NovaRED & Vector NovaRED (red) SK-4805, SK-4800	−	+	+	−	+	+		+	−
ImmPACT SG & SG (blue-gray) SK-4705, SK-4700	+	−	−	+	+	−	−		+
ImmPACT AEC, ImmPACT AMEC Red & AEC (red) SK-4205, SK-4285, SK-4200	−	−	−	−	+	−	−	+	

\+ Indicates good contrast − Indicates incompatiblity of substrates for various reasons

CITATIONS

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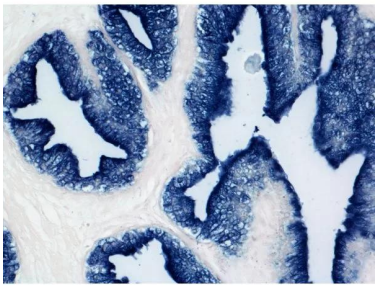
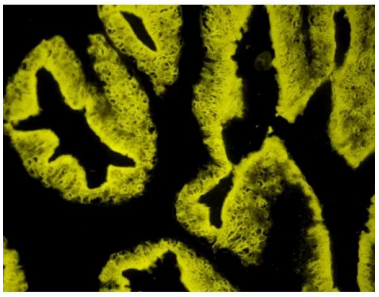
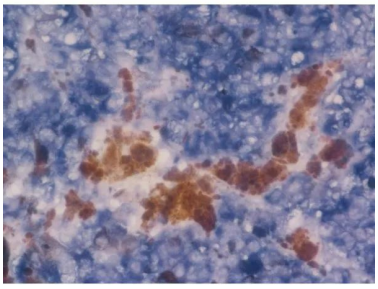
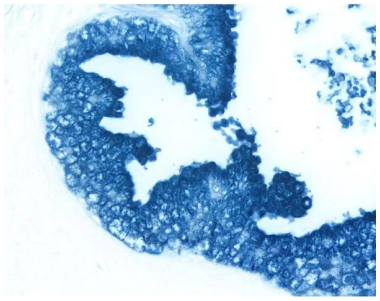
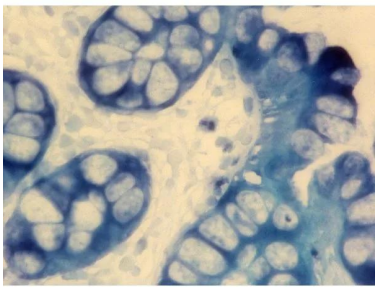
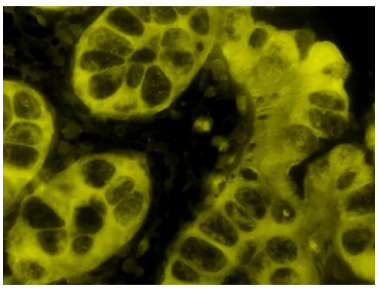
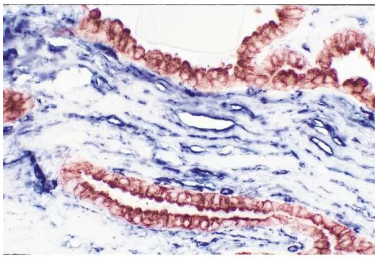
DOCUMENTS

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- Enzyme Substrates Brochure
- Enzyme Substrate Combinations Chart
- Counterstain/Substrate Compatibility Chart
- In Situ Hybridization Detection Protocol
- User Guide
- Safety Data Sheet
- Download CoA
- Datasheet

GALLERY IMAGES


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