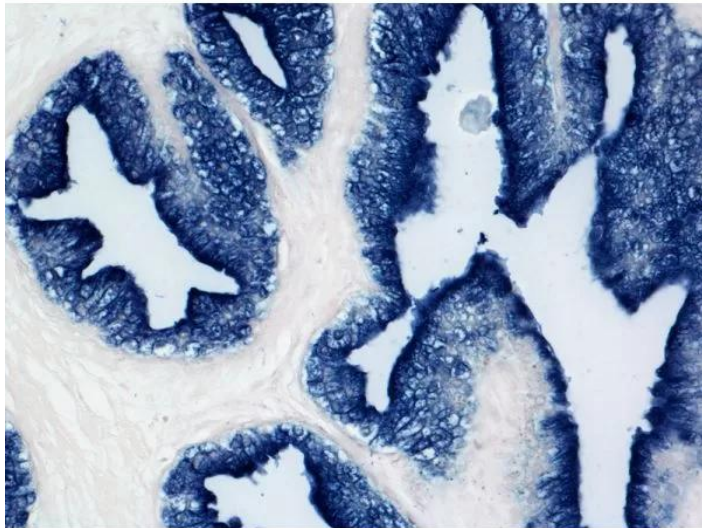




# **BCIP/NBT SUBSTRATE KIT, ALKALINE PHOSPHATASE (AP), (5-BROMO-4-CHLORO-3-INDOLYL PHOSPHATE/NITROBLUE TETRAZOLIUM)**

**SKU:** SK-5400



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## **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

|                                        |                                                                                                                       |
|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| <b>Microscopy</b>                      | Brightfield, Fluorescence, Spectral Imaging                                                                           |
| <b>Unit Size</b>                       | 1 Kit                                                                                                                 |
| <b>Applications</b>                    | Immunohistochemistry / Immunocytochemistry, Immunofluorescence, In situ hybridization, Blotting Applications, Elispot |
| <b>Additional Substrate Properties</b> | 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

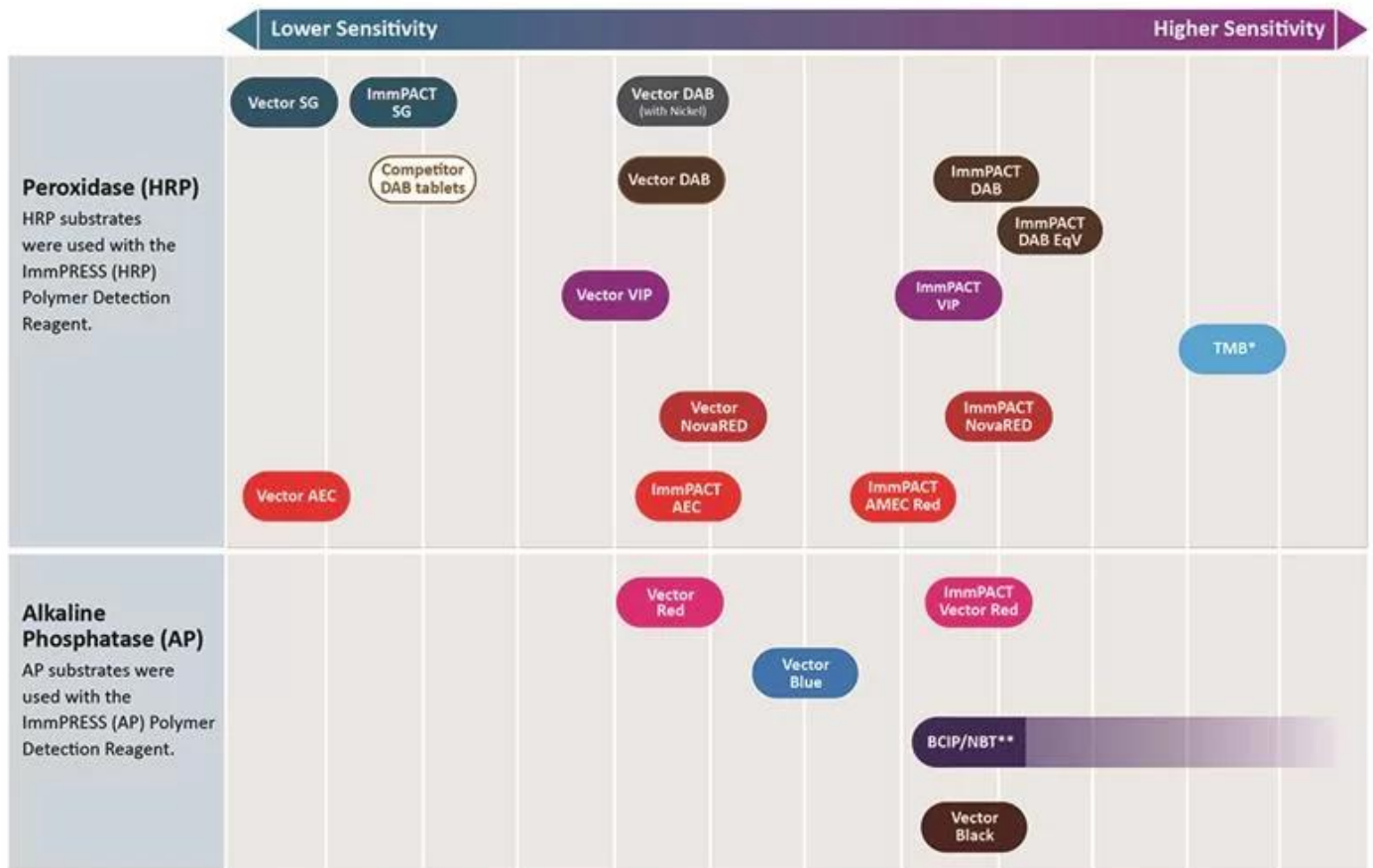
## TECHNICAL INFORMATION

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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## Relative Sensitivity of Substrates in Immunohistochemistry



\* Reaction product deposition not discreet or reliable for IHC. \*\*Longer incubation times increase sensitivity.

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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.

| Second Substrate<br>First 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

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 Indicates incompatibility of substrates for various reasons

## CITATIONS



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## DOCUMENTS

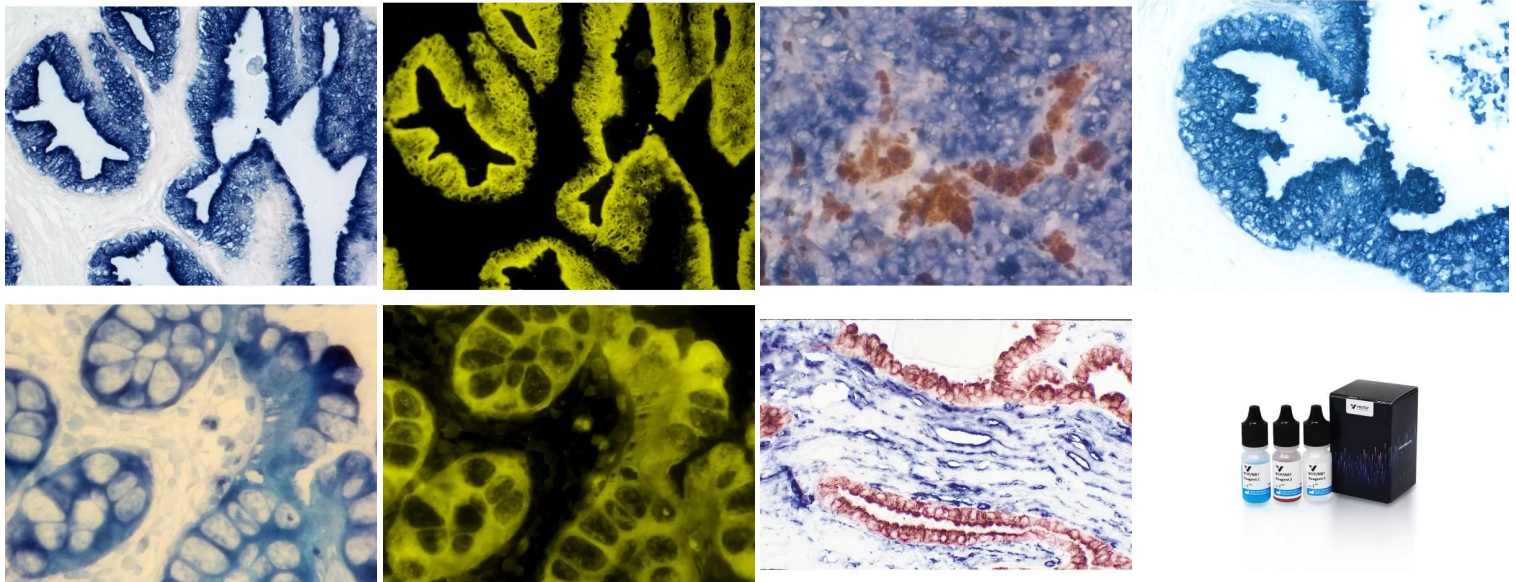
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- [Enzyme Substrates Brochure](#)
- [Enzyme Substrate Combinations Chart](#)
- [Counterstain/Substrate Compatibility Chart](#)
- [In Situ Hybridization Detection Protocol](#)
- [User Guide](#)
- [Safety Data Sheet](#)
- [Download CoA](#)
- [Datasheet](#)

## GALLERY IMAGES



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