



## **TMB SUBSTRATE KIT, PEROXIDASE (HRP), (3,3', 5,5'-TETRAMETHYLBENZIDINE)**

**SKU:** SK-4400



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### **DESCRIPTION**

TMB (3,3', 5,5' tetramethylbenzidine dihydrochloride) is a sensitive chromogen substrate for peroxidase and a non-carcinogenic substitute for benzidine. TMB can be used for blotting, immunohistochemical, and *in situ* hybridization applications. Slides stained with TMB can be dehydrated and permanently mounted and the soluble blue product, produced in the absence of the stabilizing solution, is useful for ELISA assays.

The TMB HRP Substrate Kit (SK-4400) contains the reagents needed to produce either a precipitating blue reaction product or a soluble blue reaction product. This kit also contains a stabilizing solution which, when added to the working solution, allows the development of a blue precipitate. Kit materials are pre-packaged in convenient dropper bottles.

#### **Features:**

- Greater sensitivity than conventional substrates

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- Stock solutions supplied in convenient dropper bottles promoting ease of handling
- No wait times for mixing and dissolving powders or tablets
- Sufficient reagents to produce 300 ml of working solution

## SPECIFICATIONS

|                                   |                                                                                                           |
|-----------------------------------|-----------------------------------------------------------------------------------------------------------|
| <b>Chromogen Color</b>            | Blue                                                                                                      |
| <b>Detection Enzyme</b>           | Peroxidase                                                                                                |
| <b>Microscopy</b>                 | Brightfield, Spectral Imaging                                                                             |
| <b>Mounting Medium Type</b>       | Non-Aqueous (Permanent)                                                                                   |
| <b>Unit Size</b>                  | 1 Kit                                                                                                     |
| <b>Applications</b>               | Immunohistochemistry / Immunocytochemistry, In situ hybridization, Blotting Applications, Elispot, ELISAs |
| <b>Safety Title</b>               | WARNING: Cancer – <a href="http://www.P65Warnings.ca.gov">www.P65Warnings.ca.gov</a>                      |
| <b>Substrate Reaction Product</b> | Precipitating Reaction Product                                                                            |

## KIT COMPONENTS

- 6 ml TMB Reagent 1
- 6 ml TMB Reagent 2
- 6 ml TMB Reagent 3
- 6 ml TMB Reagent 4

## TECHNICAL INFORMATION

TMB (3,3', 5,5' tetramethylbenzidine dihydrochloride) is a sensitive chromogen substrate for peroxidase and a non-carcinogenic substitute for benzidine. TMB can be used for blotting, immunohistochemical, and *in situ* hybridization applications. Slides stained with TMB can be dehydrated and permanently mounted and the soluble blue product, produced in the absence of the stabilizing solution, is useful for ELISA assays.

### Counterstain / Substrate Compatibility

This table is designed as a reference to determine the optimal counterstain / substrate combination for your application. Consideration should be given to tissue type, antigen

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unmasking protocol, and other detection parameters to achieve the desired staining intensity.

| Substrate                    | Catalog Number          | Hematoxylin (H-3404)<br>Hematoxylin QS (H-3401) | Methyl Green (H-3402)          | Nuclear Fast Red (H-3404) |
|------------------------------|-------------------------|-------------------------------------------------|--------------------------------|---------------------------|
| ImmPACT DAB (brown)          | <a href="#">SK-4105</a> | Excellent Contrast                              | Excellent Contrast             | Fair Contrast             |
| ImmPACT DAB EqV (brown)      | <a href="#">SK-4103</a> | Excellent Contrast                              | Excellent Contrast             | Fair Contrast             |
| DAB (brown)                  | <a href="#">SK-4100</a> | Excellent Contrast                              | Excellent Contrast             | Fair Contrast             |
| DAB-Ni (gray-black)          | <a href="#">SK-4100</a> | Excellent Contrast                              | Fair Contrast*                 | Good Contrast             |
| ImmPACT AEC (red)            | <a href="#">SK-4205</a> | Excellent Contrast                              | Counterstain Incompatibility** | Color Incompatibility     |
| ImmPACT AMEC Red (red)       | <a href="#">SK-4285</a> | Excellent Contrast                              | Counterstain Incompatibility** | Color Incompatibility     |
| AEC (red)                    | <a href="#">SK-4200</a> | Excellent Contrast                              | Counterstain Incompatibility** | Color Incompatibility     |
| TMB (blue)                   | <a href="#">SK-4400</a> | Color Incompatibility                           | Counterstain Incompatibility   | Excellent Contrast        |
| ImmPACT VIP (purple)         | <a href="#">SK-4605</a> | Fair Contrast                                   | Excellent Contrast             | Poor Contrast             |
| Vector VIP (purple)          | <a href="#">SK-4600</a> | Fair Contrast                                   | Excellent Contrast             | Poor Contrast             |
| ImmPACT SG (blue-gray)       | <a href="#">SK-4705</a> | Poor Contrast                                   | Good Contrast                  | Excellent Contrast        |
| SG (blue-gray)               | <a href="#">SK-4700</a> | Poor Contrast                                   | Good Contrast                  | Excellent Contrast        |
| ImmPACT NovaRED (red)        | <a href="#">SK-4805</a> | Excellent Contrast                              | Excellent Contrast***          | Color Incompatibility     |
| Vector NovaRED (red)         | <a href="#">SK-4800</a> | Excellent Contrast                              | Excellent Contrast***          | Color Incompatibility     |
| ImmPACT Vector Red (magenta) | <a href="#">SK-5105</a> | Excellent Contrast                              | Excellent Contrast             | Color Incompatibility     |
| Vector Red (magenta)         | <a href="#">SK-5100</a> | Excellent Contrast                              | Excellent Contrast             | Color Incompatibility     |

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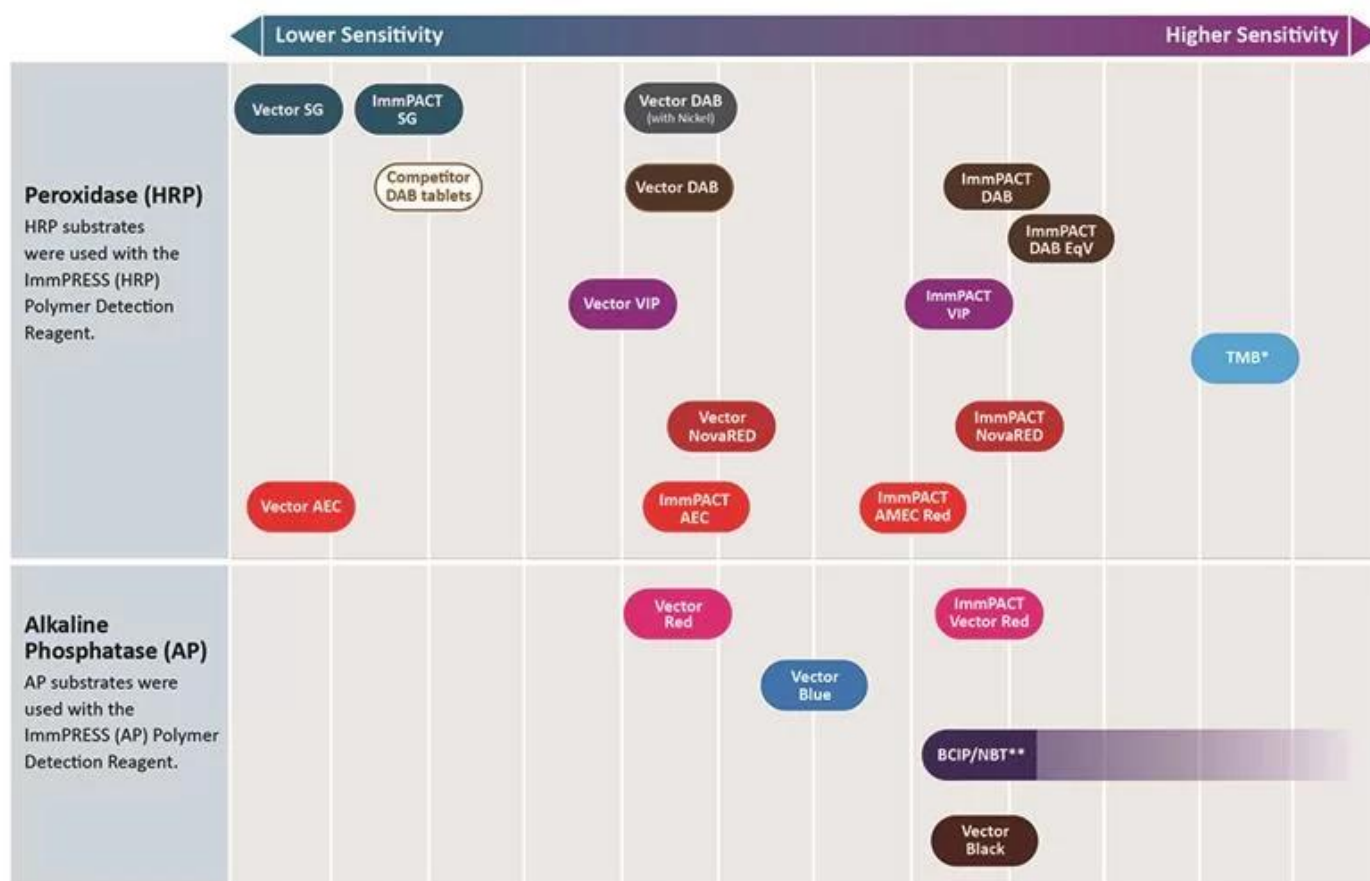
|                      |                         |                       |                     |                    |
|----------------------|-------------------------|-----------------------|---------------------|--------------------|
| Vector Black (black) | <a href="#">SK-5200</a> | Excellent Contrast    | Excellent Contrast* | Excellent Contrast |
| Vector Blue (blue)   | <a href="#">SK-5300</a> | Color Incompatibility | Good Contrast       | Excellent Contrast |
| BCIP / NBT (indigo)  | <a href="#">SK-5400</a> | 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.

\*\*Substrate dissolves in acetone wash.

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

### Relative Sensitivity of Substrates in Immunohistochemistry



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

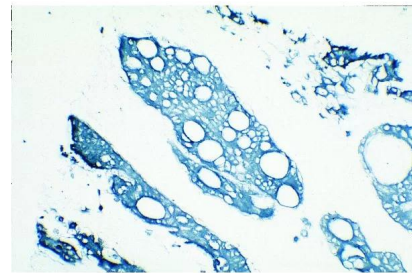
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## DOCUMENTS

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

## GALLERY IMAGES



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