

TrueBlue™

## TrueBlue™ Peroxidase Substrate for Immunohistochemistry

TrueBlue is a highly sensitive chromogenic substrate for visualization of horseradish peroxidase-labeled reporter reagents. It provides a brilliant blue specific stain, which contrasts sharply with red counterstains. TrueBlue Substrate is a buffered solution containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) and Hydrogen Peroxide ( $H_2O_2$ ). TrueBlue offers the following benefits:

### Highest Sensitivity

TrueBlue is 50 to 100 times more sensitive than DAB. It provides an excellent means for detecting low abundant target antigens.

### Safe-to-Use

TrueBlue is non-carcinogenic and safe-to-use, no special handling and disposal required.

### Economical

TrueBlue permits extremely high dilutions of costly primary antibodies. You can perform many more assays with TrueBlue, saving you time and money. This stable, liquid system eliminates waste and reduces preparation time.

### Ideal for Multiple Staining

TrueBlue forms a dark blue reaction product that offers outstanding contrast with DAB and other substrates in sequential localization assays.

### Highly Stable and Convenient

TrueBlue is provided as a stable, single-component working solution that is ready-to-use. No reagent preparation required.



Power Your  
Immunoassays

## Technical Tips

- Primary antibodies must be diluted 10 to 50 times further than with DAB, otherwise sample will over-stain. Excess substrate may wash off the section, causing the appearance of understaining.
- Color development in less than 10 minutes indicates that the primary antibody is too concentrated.
- Blocking is essential because TrueBlue is extremely sensitive. Trace levels of peroxidase and non-specific binding sites in the tissue must be neutralized to prevent background staining.
- Peroxidase block solution must be thoroughly removed by washing; it can affect antibody activity.
- KPL recommends non-aqueous mounting reagent. Fading will occur if aqueous media are used.
- Stained sections must be completely dry or color may fade rapidly after mounting.

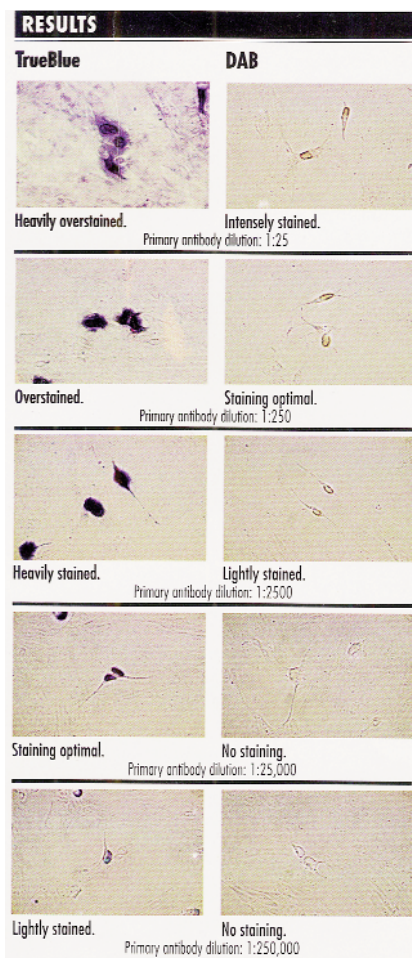
## TrueBlue Comparison to DAB

### Methodology

TrueBlue and DAB were compared using increasing dilutions of monoclonal antibody to latent cytomegalovirus (CMV) antigen on CMV-infected, acetone-fixed fibroblast cell monolayers. Monolayers were immunostained using identical protocols (monoclonal antibody and conjugate incubations of 30 minutes each, and substrate incubations of 10 minutes each).

### Conclusion

TrueBlue exhibited 100 times higher sensitivity than DAB. When using TrueBlue, dilute primary antibodies 10 to 50 times further for superior staining and lower background.



## Ordering Information

Catalog No.	Description	Size
50-78-02	TrueBlue Peroxidase Substrate	200 mL
71-00-64	TrueBlue Peroxidase Substrate	50 mL
71-00-67	TrueBlue Peroxidase Substrate	10 mL

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