KPL HistoMark® BLUE

For Localization of Alkaline Phosphatase-Labeled Reagents

Catalog No.	Size
5510-0037 (55-70-00)	500 mL

DESCRIPTION

KPL HistoMark® BLUE Substrate System is designed for visualization of alkaline phosphatase-labeled (AP) reagents. KPL HistoMark BLUE is a Fast Blue stain and KPL Contrast RED is a nuclear counterstain formulated from nuclear Fast Red. The substrate system provides a blue (Fast Blue) specific stain with red counterstain for immunohistochemical staining or immunoblotting.

KIT COMPONENTS

	Catalog No.	Volume
KPL PhThaloBLUE	5510-0039 (71-00-03)	10 mL
KPL Activator Solution	5570-0002 (71-00-01)	10 mL
KPL Buffered Substrate	5570-0003 (71-00-04)	50 mL
KPL Contrast RED	5540-0001 (71-00-05)	50 mL

Sufficient reagents are supplied to prepare 500 mL Substrate Solution (approximately 1000 slides).

STORAGE/STABILITY

Reagents are stable for a minimum of one year stored at 2-8°C.

Store KPL Contrast RED Solution tightly capped at room temperature.

Discard KPL PhThaloBLUE Solution if black precipitate develops.

Discard KPL Activator Solution or KPL Buffered Substrate Solution if yellow color develops.

Warm all reagents to room temperature (24-28°C) before use.

If a light precipitate is visible in KPL Buffered Substrate Solution, warm for 10 - 30 minutes in 37°C waterbath. Mix thorough

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10. Wash as in Step 6. (Prepare KPL Substrate Solution during this step.)
11. Shake off excess buffer and cover section with KPL Substrate Solution.
12. Incubate 10 minutes at room temperature out of direct light.
13. Rinse slide 2 - 3 minutes in reagent quality water.
14. Counterstain in KPL Contrast RED Solution 5 - 10 minutes.
15. Rinse thoroughly in reagent quality water until excess stain is removed from slide.
16. Air dry and mount in aqueous mounting medium. DO NOT USE XYLENE BASED MOUNTING MEDIA.

RESULTS

Nuclei appear a contrasting pale pink to red.

negative control should not develop any blue tint.

NOTES

1. Always incorporate appropriate positive and negative controls.
2. Use substrate reagents immediately after mixing.
3. Instant development of blue color indicates that the primary antibody or phosphatase-labeled reagent must be further diluted.
4. Prolonged incubation in substrate may increase background and inhibit nuclear counterstaining.

BUFFER PREPARATION

0.1 M Tris-HCl

a. Dissolve 121 g Tris in 500 mL reagent quality water.
b. Adjust pH to 7.6 with 2 M HCl (approximately 300 mL).
c. QS to 1 L with reagent quality water to obtain a 1 M stock.
d. Dilute 1 part stock from Reagents Section, Step 5, with 9 parts reagent quality water and mix well.

1 M Citric Acid Free Acid

a. Dissolve 192 g of citric acid free acid in 500 mL reagent quality water.
b. QS to 1L with reagent quality water.

PRINCIPLE

The application of antibodies and other reagents such as avidin, streptavidin, etc., covalently coupled to calf intestine alkaline phosphata-5(t)-5(h-6()4(t)5ETBT1 0 0 2Etd o4 1