Diagnostic BioSystems

IHC Made Affordable

PermaBlue/AP

K 051
K 051-110

Document #: DS-4002-A
Effective Date: 04/01/2015

Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
PermaBlue/AP is a substrate-chromogen system designed to be used for either IHC or ISH when utilizing alkaline phosphatase. PermaBlue/AP produces a vibrant blue color. PermaBlue/AP is insoluble in alcohol and xylene substitutes (both aliphatic hydrocarbon and citrus based); therefore sections can be dehydrated in alcohol, cleared in xylene substitute, and permanently mounted.

Principles of the Procedures
Substrate/chromogen in conjunction with alkaline phosphatase (AP)-based immunostaining or in situ hybridization systems.

Reagents Provided

Kit Contents	30 mL	110 mL
PermaBlue/AP Substrate Buffer	30 mL	110 mL
PermaBlue/AP Chromogen	1 mL	3 mL
Empty Mixing Bottle	1	1

Prepare the Following Solutions Before Use
1. Aliquot 1mL of PermaBlue/AP Substrate Buffer in a mixing bottle.
2. Add one drop (~20µl) of concentrated PermaBlue/AP Chromogen solution.
3. Replace tip, mix, and allow solution to reach room temperature before using.
4. The PermaBlue/AP chromogen-substrate working solution should be used within 25 minutes of preparation.
5. Any solution not used during this period should be discarded.

Materials Required But Not Provided
Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com.

Storage and Handling
Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure
1. Once sections have been incubated with alkaline phosphatase, wash tissue sections with wash buffer.
2. Wipe slides, removing excess buffer.
3. Add enough drops of PermaBlue/AP working solution to cover tissue sections.
4. Incubate for 5-25 minutes at room temperature.

Notes: For optimal results, observe reaction under microscope for signal development. Once desired signal to noise ratio is achieved, stop reaction by rinsing slides with DI H2O.
5. Increasing incubation temperature to 37˚C will increase sensitivity and decrease needed incubation time.
6. Counterstain. Nuclear Fast Red provides good contrast. Wash with DI H2O.
7. Dehydrate sections in alcohol, clear in a xylene-substitute*, and mount in permanent mounting medium.
8. Use increasing concentrations of Ethanol up to 100% to dehydrate.
9. Alternatively, slides can be air dried (instead of dehydrated or cleared in alcohol and xylene-substitute).
10. After rinsing off counterstain in DI H2O, leave slides on benchtop for at least 20 minutes to air dry, and then permanently mount.
Notes: Use xylene-substitute instead of xylene.

Precautions
1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.

Troubleshooting
If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

Diagnostic BioSystems
6616 Owens Drive
Pleasanton, CA 94588
Tel: (925) 484 3350
www. dbiosys.com

CE

EC REP

Emergo Europe
Molenstraat 15
2513 BH, The Hague
The Netherlands
Tel: (31) (0) 70 345 8570