Diagnostic BioSystems

IHC Made Affordable

PermaRed/AP

K 049
K 049-110

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
PermaRed/AP is a substrate-chromogen system designed to be used for either IHC or ISH when using alkaline phosphatase detection. PermaRed/AP produces a brilliant dark red color. PermaRed/AP is insoluble in organic solvents; therefore, sections can be dehydrated in alcohol, cleared in xylene (or a xylene-substitute), and permanently mounted. This chromogen substrate system may be used for both automation and manual use.

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

Reagents Provided

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

Prepare the Following Solutions Before Use
1. Aliquot 1mL of PermaRed/AP Substrate Buffer in a mixing bottle.
2. Add one drop (~20µl) of concentrated PermaRed/AP Chromogen solution.
3. Replace tip, mix, and allow solution to reach room temperature before using.
4. The working chromogen-substrate solution should be prepared fresh and used within 20-30 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 sections with wash buffer then follow protocol of choice:
   a. **Batch Mode: (Automation)** Using Batch Mode on your instrument, wait for machine to notify you when ready, then mix chromogen and buffer in a 1:50 ratio and load onto instrument. Working solution is stable for only 20-30 minutes and should be applied to slide immediately for best results. Incubate for 10 - 20 minutes.
   b. **On Board Mixing: (Automation)** Instruments that have on-board mixing capability can load the chromogen and substrate-buffer components independently. Working solution is made mixing reagents 1:50 using on-board mixing station before application to slide. Incubate for 10 - 20 minutes.
   c. **Manual Use** Mix substrate-chromogen and buffer in a 1:50 ratio and apply directly to slide. Incubate for 10 – 20 minutes.
2. Counterstain with Hematoxylin or other counterstain.
3. Wash with DI H2O followed by immuno wash buffer.
4. Sections can be dehydrated in alcohol, cleared in xylene or xylene substitute and permanently mounted.

Note: Alternatively, slides can be air dried (instead of alcohol and xylene). After rinsing off counterstain in DI H2O, leave slides on benchtop for at least 20 minutes to air dry, then permanently mount or use aqueous mounting media (DBS Cat# CC Mount K002).

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.

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