Diagnostic BioSystems

IHC Made Affordable

# PermaRed/AP-Auto
# (For Use on Automated Staining Platforms)

K 049 Auto
K 049-Auto-110

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**Intended Use**

For In Vitro Diagnostic Use

**Summary and Explanation**

Traditional formulations for red alkaline phosphatase chromogens are unstable upon mixing. This can be problematic for use in automated slide strainers. PermaRed/AP-Auto has been formulated for on-slide and on-board mixing for any autostainer. Both components are stable and can be loaded at the beginning of the staining run, for walk away convenience.

PermaRed/AP-Auto produces a red color, ranging from pink to brilliant dark red. It 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**

PermaRed/AP-Auto is a substrate/chromogen system for use with alkaline phosphatase (AP) detection in immunohistochemistry (IHC) or in situ hybridizations (ISH) run on automated staining instruments.

**Reagents Provided**

| Kits Contents | 30 mL | 110 mL |
|---|---|---|
| PermaRed/AP Substrate Buffer | 30 mL | 110 mL |
| PermaRed/AP Chromogen | 30 mL | 110 mL |

**Prepare the Following Solutions Before Use**

1. PermaRed/AP Auto 1X Working Solution: In a clean dark bottle, mix equal volumes of PermaRed/AP Auto Buffer and PermaRed/AP Auto Chromogen in a 1:1 ratio.
2. MAKE FRESH: WORKING SOLUTION IS STABLE FOR ONLY 20-30 MINUTES
3. There is no preparation required for automation use. Reagents can be loaded directly on the instrument for on-slide mixing or on-board mixing.

**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.
   a. **On-Slide Mixing (Automation):** PermaRed/AP-Auto has been formulated for direct application of the component solutions onto the slide in a specified order: sequential application of substrate buffer first, followed by chromogen in equal volumes with no washing or reagent "blow off" in between. Reversing the order of reagent application will increase background and yield suboptimal results. Incubate Substrate Buffer for 1-5 minutes. Incubate Chromogen for 10-20 minutes.
   b. **Batch Mode (Automation)**: Using Batch Mode on your instrument, wait for machine to notify you when ready, then mix chromogen and buffer in a 1:1 ratio and load onto instrument. Incubate working solution for 10 – 20 minutes.
   c. **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 1:1 in on-board mixing station before application to slide. Incubate working solution for 10 – 20 minutes.
   d. **Manual Use:** Mix chromogen and buffer in a 1:1 ratio and apply directly to slide. Working solution is stable for only 20-30 minutes and should be applied immediately for best results. Incubate working solution for 10 – 20 minutes.
2. Counterstain with Hematoxylin for good contrast. Wash with DI H2O followed by immuno wash buffer.
3. Sections can be dehydrated in alcohol, cleared in xylene or xylene substitute and permanently mounted.
4. 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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