PermaRed/AP-Auto

For Use on Automated Staining Platforms

Catalog No. K 049-110-AUTO

Intended Use 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.

Description Traditional formulations for red alkaline phosphatase chromogens are unstable upon mixing. These working solutions must be used fresh. After 15 minutes has elapsed, the color development drastically decreases. This can be problematic for use in automated slide stainers without on-board reagent mixing stations (e.g. DAKO, Lab Vision, Biogenex). The user must return to the instrument to add freshly made working solution. Often, the 15 minute reaction window will be exceeded before the substrate/chromogen is applied to the slide due to program delays for washes and timing pauses. This leads to poor and inconsistent staining.

PermaRed/AP-Auto has been formulated for on-slide mixing. The instrument should be programmed to apply the substrate-buffer to the slide followed by application of the chromogen reagent. The substrate-buffer and chromogen components are stable in their separate formats. They can be loaded into the reagent rack at the beginning of the staining run with the other reagents, for walk away convenience.

PermaRed/AP-Auto produces a red color, ranging from pink to brilliant dark red, depending on the signal intensity. It is insoluble in organic solvents; therefore sections can be dehydrated in alcohol, cleared in xylene (or a xylene-substitute), and permanently mounted.

Components
1) 110mL PermaRed/AP-Auto Substrate Buffer
2) 110mL PermaRed/AP-Auto Chromogen

Storage Store components at 2-8°C. Do not use beyond expiration date stated on the label.

Important Notes PermaRed/AP-Auto has been formulated for direct application of the component solutions onto the slide in a specified order: Substrate Buffer first, followed by Chromogen. Preparation of a pre-mixed working solution is NOT required, and will yield suboptimal staining. Reversing the order of reagent application will increase background and yield suboptimal results.

The program software on the automated staining platform must allow sequential application of the substrate-buffer component followed by the chromogen component with no washing or reagent "blow off" in between.

The reagents are formulated to be applied to the slide sequentially, in equal volumes. The component reagents can be applied manually to slides dropwise, adding Substrate-Buffer first, followed by an equal number of Chromogen drops.

Contact DBS Technical Support for detailed assistance with protocols.

Precautions Wear appropriate personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required. Interpretation of the results is the sole responsibility of the user

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STEP	STAINING PROCEDURE:	INCUBATION TIME
1. Dewaxing and Rehydration	Follow the standard dewaxing and rehydration protocol used in your lab.	
2. Epitope Recovery	Follow the recommended epitope recovery method recommended for your tissue and antigen(s).	
Rinse (Buffer)		
3. Endogenous Enzyme Block	Incubate with Pre-Blocking Solution, if desired.	**10 min.**
Rinse (Buffer)		
4. Primary Antibody	Incubate with Primary Antibody, prepared according to the manufacturer's recommended protocol at the desired concentration.	**30 – 60 min.**
Rinse (Buffer)		
5. Secondary Reagent	Incubate with secondary detection reagent (e.g. anti-Mouse/anti-Rabbit Linker, Polymer Penetration Enhancer reagent, etc.).	**10-20 min.**
Rinse (Buffer)		
6. Tertiary Reagent	Incubate with AP-detection reagent (e.g. Streptavidin-AP Tracer, Mouse/Rabbit AP polymer reagent, etc.).	**10-20 min**
Rinse (Buffer)		
7. Substrate	Incubate with **PermaRed/AP-Auto Buffer Substrate.**	**1-10 min.**
8. Substrate	Incubate with **PermaRed/AP-Auto Chromogen.** **(**If stronger signal is desired, increase chromogen incubation time; for less intense signal, decrease chromogen incubation time)	**5 - 15 min.**
Rinse (Water)		
9. Auxiliary	Incubate with Counterstain (e.g. Hematoxylin)	**1 min**
Rinse (Water)		
Rinse (Buffer)		
Rinse (Water)		
10. Mount Coverslips	Stained tissue sections can be dehydrated in alcohol and cleared in xylene or xylene substitute and permanently mounted.	

IVD: For In Vitro Diagnostic Use

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