ab176915

StayBlue/AP Plus Stain Kit

Instructions for Use

An immunohistochemical chromogen substrate for staining tissue sections

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 4 February 2019

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1. Introduction

StayBlue/AP Plus is a substrate chromogen system designed to be used for either IHC or ISH when using alkaline phosphate detection. StayBlue/AP Plus produces a blue color. It 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. This chromogen substrate system may be used for both automation and manual use.

2. Kit Contents

Components	Amount		Storage
	30 ml	110 ml	
StayBlue/AP Plus Substrate Buffer	30 ml	110 ml	4°C
StayBlue/AP Plus Chromogen	1 ml	3ml	4°C
Empty Mixing Bottle	1	1	RT

3. Storage and Handling

Protect reagents from light and store as given in the Table.

4. Preparation of Working Solution

Aliquot 1 ml of StayBlue/AP Plus Substrate Buffer into a mixing bottle. Add one drop (~20 µl) of Concentrated StayBlue/AP Plus Chromogen solution. Replace tip, mix, and allow solution to reach room temperature before using.

Note: StayBlue/AP Plus chromogen-substrate Working Solution is light sensitive and should be kept away from light as much as possible.

Working Solution is stable for up to 6 hours in the dark. Any solution not used during this period should be discarded.

5. Staining Protocol

A. Manual Method:

1. Following alkaline phosphatase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer.
3. Add enough drops of StayBlue/AP Plus Working Solution to cover tissue sections.
4. Incubate for 10-20 minutes at room temperature.

   For optimal results, observe reaction under the microscope for signal development. Once the desired signal to noise ratio is achieved, stop the reaction by rinsing the slides with DI H_2O.

B. Automated Method using Pre-Mixed Working Solution:

1. Following alkaline phosphatase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer.
3. StayBlue/AP Plus working solution is stable for 6 hours and can be loaded directly onto instrument as a single solution. Reduce exposure to light to achieve optimal staining.
4. Apply Working Solution directly to slide.
5. Incubate for 10-20minutes at room temperature.

C. Automated Method using On Board Mixing:

1. Following alkaline phosphatase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer.
3. Instruments that have on-board mixing capability can load the chromogen and substrate-buffer components independently. Working Solution is made mixing reagents 1:50 in on-board mixing station before application to slide.
4. Apply Working Solution directly to slide.
5. Incubate for 10-20 minutes at room temperature

D. Counterstain:

Counterstain with Hematoxylin or Nuclear Fast Red for good contrast. Wash with DI H_2O followed by immuno wash buffer.

E. Mounting:

1. Dehydrate sections in alcohol, clear in a xylene-substitute*, and mount with a permanent mounting medium.
   *Notes:
   Use increasing concentrations of Ethanol up to 100% to dehydrate.
   Use xylene-substitute instead of xylene.

2. Alternatively, slides can be air dried (instead of dehydrated or cleared in alcohol and xylene-substitute). After rinsing off counterstain in DI H_2O, leave slides on benchtop for at least 20 minutes to air dry, and then permanently mount.

6. Reference Image

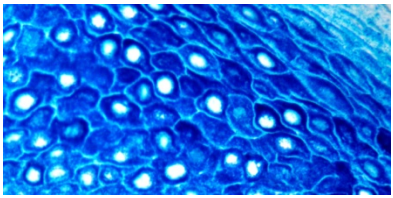

Figure 1 Formalin-fixed; paraffin-embedded Human tonsil tissue stained with HMW CK antibody labeled with StayBlue/AP Plus (ab176915) produces a strong blue color.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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