ab179917

StayBlack/HRP 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 1 Last Updated 1 February 2019

Table of Contents

1. Introduction

StayBlack/HRP is a substrate chromogen system designed to be used for either IHC or ISH when using horseradish peroxidase detection. StayBlack/HRP produces a black color. This chromogen substrate system may be used for both automation and manual use.

2. Kit Contents

Components	Amount		Storage
	30 ml	**110 ml**	
StayBlack/HRP Substrate Buffer	30 ml	110 ml	4°C
StayBlack/HRP 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 StayBlack/HRP Substrate Buffer into a mixing bottle. Add one drop (~20 µl) of Concentrated StayBlack/HRP Chromogen solution. Replace tip, mix, and allow solution to reach room temperature before using.

Note: StayBlack/HRP 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 1 day in the dark. Any solution not used during this period should be discarded. For optimal results prepare fresh reagent.

5. Staining Protocol

A. Manual Method:

1. Following peroxidase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer.
3. Add enough drops of StayBlack/HRP Working Solution to cover tissue sections.
4. Incubate for 5-10 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 peroxidase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer.
3. StayBlack/HRP working solution is stable for at least 1 day 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 5-10minutes at room temperature.

C. Automated Method using On Board Mixing:

1. Following peroxidase 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 5-10 minutes at room temperature

D. Counterstain:

Counterstain with Hematoxylin or other counterstain. 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

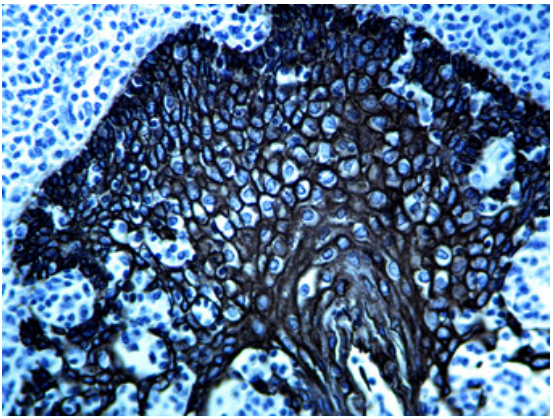

Figure 1 Formalin-fixed; paraffin-embedded Human tonsil tissue stained with HMW CK antibody labeled with StayBlack/HRP (ab179917) produces a strong black color.

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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