Stayright™ Purple HRP Staining Kit

Catalog number: 45905, 45906
Unit size: 5 mL, 50 mL

Component	Storage	Amount (Cat No. 45905)	Amount (Cat No. 45906)
Component A: 100X Stayright™ Purple	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (50 µL)	1 bottle (500 µL)
Component B: Stayright™ Purple HRP buffer	Freeze (< -15 °C)	1 bottle (5 mL)	1 bottle (50 mL)
Component C: Stabilized 3% Hydrogen peroxide (H_2O_2)	Freeze (< -15 °C)	1 vial (10 µL)	1 bottle (100 µL)

OVERVIEW

3,3'-Diaminobenzidine (DAB) has been applied for decades as the most commonly used IHC chromogen because it is inexpensive and sensitive for routine applications. However, DAB has been shown to be mutagenic and hazardous to laboratory workers and the environment. In order to address this issue, AAT provides Stayright™ Purple as a significantly safer IHC chromogen than DAB. Furthermore, Stayright™ Purple provides a rapid and simple method to develop clean and intense purple stain in the presence of HRP with high sensitivity as DAB. The Stayright™ Purple HRP substrate also shows non-mutagenic effects with minimal cytotoxicity. ReadiUse™ Stayright™ Purple Peroxidase (HRP) Substrate is suitable for use in peroxidase (HRP)-based immunohistochemistry (IHC) and in situ hybridization (ISH) staining methods. Upon HRP-induced oxidation, Stayright™ Purple forms a purple insoluble precipitating product at the target site of your assay. The purple end product is insoluble in organic solvents and organic mounting media, thus the distinct purple stain can maintain through regular dehydration and coverslipping steps. For enhanced convenience, you try our ReadiUse™ Stayright™ Purple Peroxidase (HRP) Substrate (#45900 and 45901). It is a stable pre-mixed solution containing hydrogen peroxide so all mixing steps are eliminated and is ready to use.

AT A GLANCE

Protocol Summary

1. Apply the Stayright™ Purple working solution to the tissue section.
2. Incubate the tissue section for 5 to 15 minutes.
3. Rinse the tissue sections for 5 to 10 minutes, then if needed proceed with counterstaining.
4. Apply the mounting medium to cover the section.

KEY PARAMETERS

Light microscope

Instrument specification(s)	White light

PREPARATION OF WORKING SOLUTION

Stayright™ Purple Working Solution

1. Prepare a Stayright™ Purple working solution by adding 10 µL of 100X Stayright™ Purple (Component A) and 1 µL of stabilized 3% Hydrogen peroxide (Component C) to every 1 mL of Stayright™ Purple HRP buffer (Component B).

Note: Unused pre-mixed working solution can be stored at 2-4°C for a few weeks. However, we recommend preparing the mixture fresh for each application.

SAMPLE EXPERIMENTAL PROTOCOL

1. Apply the Stayright™ Purple working solution to the tissue section and let it incubate at room temperature for 5 to 15 minutes.
2. Immerse the slides in dH2O to stop the color development process. Monitor the staining intensity carefully. If the staining intensity is insufficient, a longer incubation period is needed. You can reapply the Stayright™ Purple working solution to further develop the staining.
3. Wash the tissue sections with dH2O for 5 to 10 minutes.
4. Use a desired counterstain if necessary.
5. Dehydrate the specimen using ethanol and then mount it permanently in an organic mounting medium.

Note: For guidelines on sample preparation, please visit

https://www.aatbio.com/resources/guides/paraffin-embedded-tissue-immunohistochemistry-protocol.html

EXAMPLE DATA ANALYSIS AND FIGURES

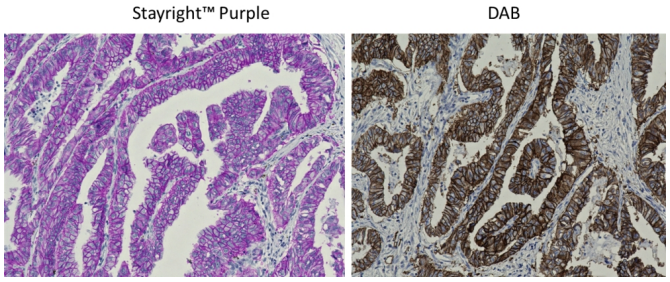

Figure 1. Immunohistochemical detection of EpCAM in FFPE lung adenocarcinoma tissue. The tissue sections were incubated with poly-HRP conjugated Goat anti-Rabbit IgG and then developed with Stayright™ Purple (Left) or DAB (Right), respectively. Cells were also counterstained with hematoxylin. Stayright™ Purple generates an intense stain with high sensitivity and clear resolution similar as DAB.

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