

## Stayright™ Purple

Catalog number: 45905, 45906

Unit size: 5 mL, 50 mL

Component	Storage	Amount	
		Cat No. 45905	Cat No. 45906
Component A: 100X Stayright™ Purple	Refrigerate (2-8 °C), Minimize light exposure	1 bottle (50 µL)	1 bottle (500 µL)
Component B: Stayright™ Purple HRP buffer	Refrigerate (2-8 °C)	5 mL	50 mL
Component C: Stabilized 3% Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	Refrigerate (2-8 °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 working Stayright™ Purple solution to tissue section.
2. Incubate tissue section for 5-15 minutes.
3. Rinse tissue for 5-10 minutes, counterstain.
4. Add mounting medium to cover the section.

### KEY PARAMETERS

Instrument: Light microscope  
 Instrument specification(s): White light

### PREPARATION OF WORKING SOLUTION

Add 10 µL of 100X Stayright™ Purple (Component A) and 1 µL of stabilized 3% Hydrogen peroxide (Component C) in every 1 mL of Stayright™ Purple HRP buffer (Component B).

**Note** Unused pre-mixed working solution can be stored for few weeks in 2-4 °C for future application. However, we recommend mixing all these components as needed.

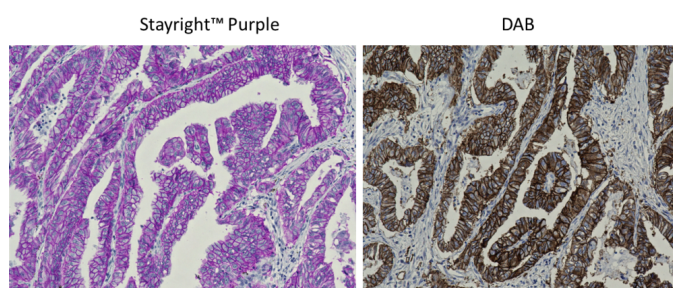
### SAMPLE EXPERIMENTAL PROTOCOL

1. Cover section with the working Stayright™ Purple solution. Incubate at room temperature for 5-15 minutes.
2. Immerse slides in dH<sub>2</sub>O to stop the color development and monitor the staining intensity. If the staining intensity is not bright enough, longer incubation is needed. One can re-apply the Stayright™ Purple solution to continue the development.

3. Wash with dH<sub>2</sub>O for 5-10 minutes.
4. Use a desired counterstain if needed.
5. Dehydrate with ethanol and permanently mount in organic permanent 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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