N-Histofine®
DAB-3S kit

Peroxidase Chromogen/Substrate kit

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Reagent supplied : 500 tests **Code : 415192F**
1500 tests **Code : 415194F**

3,3'-DIAMINOBENZIDINE TETRAHYDROCHLORIDE (DAB) SUBSTRATE KIT

SUBSTRATE FOR HORSERADISH PEROXIDASE

FORM

Liquid.

This product contains the following reagents;		[500 tests]	[1500 tests]
Reagent A : DAB solution concentrate	…	3mL × 1 bottle	9mL × 1 bottle
Reagent B : Enhancer solution concentrate	…	3mL × 1 bottle	9mL × 1 bottle
Reagent C : Hydrogen peroxide solution concentrate	…	3mL × 1 bottle	9mL × 1 bottle
Dropping bottle	…	1 bottle	1 bottle

PREPARATION of Chromogen/Substrate solution

1.Add 1 drop of DAB solution concentrate(Reagent A) to 1 mL of distilled water into the tube. Mix well.
2.Add 1 drop of Enhancer solution concentrate (Reagent B) to 1 mL of diluted Reagent A solution. Mix again.
3.Add 1drop of Hydrogen peroxide solution concentrate (Reagent C) to 1 mL of diluted Reagent A and Reagent B solution. Mix again.
4. The final mixture is stable for 14 days at 2-8°C with light shielding*.

*: Though the final mixture is stable for 14 days at 2-8 °C with light shielding, the mixture may result in a precipitate with time. The precipitation will have no effect on staining. However, in some cases, some precipitation attached on slides or tissues, may disturb getting crisp image. To avoid such precipitation attachment, following procedures are recommended.

1. Use supernatant of the mixture
2. Rinse the slides in PBS containing 0.1% Tween 20 or TBS containing Tween 20 at room temperature for three minutes, in twice, before and after the step of the mixture reaction described in USAGE.

Note regarding Dropping bottle

1) The dropping bottle may be suitable for dropping the final mixture on the slides, not for mixing the reagents, nor for storage of the final mixture.
2) If the final mixture is moved from the tube to the dropping bottle, careful handling (e.g. using pipette) is required.
3) If the remaining final mixture is stored, it shall be moved from the dropping bottle to appropriate vessels (e.g. sealed bottles or tubes) and stored at 2 – 8 °C with light shielding.
4) Rinse the dropping bottle thoroughly with distilled water after use and dry it enough prior to reuse.

USAGE

This substrate will produce a brown colored deposit upon reaction with peroxidase.

For color development, incubate the tissue section, which has been reacted with peroxidase conjugate, with 1-2 drops of the final mixture at room temperature for 5-20 minutes. Color development may be monitored with a microscope. After adequate coloration, wash the slide with tap water.

Note regarding the final mixture : Use the final mixture after adjusting to room temperature.

This brown deposit is insoluble in organic solvent. Therefore, the slide can be mounted with permanent mounting media after washing with water, dehydrated in graded series of alcohol and cleared in xylene.

STORAGE

Store at 2-8°C.

REMARKS

For research use only. Not for diagnostic use.

WARNING !

1.Reagent A (DAB solution concentrate) should be handled with care for it contains carcinogen.
2.Reagent C (Hydrogen peroxide solution concentrate) should be handled with care for it contains Hydrogen peroxide.

01/2008