Stable DAB/Plus

K047

DAB is a widely used chromogen for immunoperoxidase staining and is well-accepted among pathologists because of its increased sensitivity and ability to give cleaner background as compared to amino ethylcarbazole (AEC). Specimens stained in DAB can be dehydrated, cleared, and mounted for permanent record keeping. Stable DAB/Plus is more sensitive and stable than traditional working DAB solutions.

Principles of the Procedures

Substrate/chromogen in conjunction with peroxidase-based immunostaining systems.

Stable DAB/Plus offers several noteworthy improvements and benefits as compared to traditional working DAB solutions. Stable DAB/Plus is much more sensitive, providing a cost-effective option of diluting the primary antibody. Instead of the typical six hours for traditional DAB working solutions, Stable DAB/Plus is stable for five days, allowing users the convenience of making one working solution for the entire work week. Hazardous waste generation from spent DAB solution is also significantly reduced. In addition, Stable DAB/Plus is ideal for high volume labs and automated stainers.

Peroxidase from the antibody detection system reacts with H_2O_2 substrate to degrade it, which then reacts with DAB, precipitating it at positive sites yielding a dark brown color.

I. Reagents Required

Reagent	Volume
Concentrated, Amber-Colored DAB Chromogen Solution	5 mL
Clear Stable DAB/Plus Substrate Buffer	200 mL
Empty Mixing Dropper Bottle	1 bottle

II. Materials Required but Not Supplied

Some of the reagents and materials required for IHC are not provided. Visit www.rockland.com for available IHC supporting reagents.

III. Prepare the Following Solutions Before Use

1. Aliquot 1mL of Stable DAB/Plus Buffer in mixing bottle.
2. Add 20µL (one drop) of concentrated Stable DAB/Plus Chromogen. Replace tip and mix.
3. The working Stable DAB/Plus solution is stable for at least 5 days and should be prepared in an opaque bottle.
4. Store at 2-8°C when not in use.
5. Any solution not used after this period should be discarded

IV. Staining Procedure

1. After peroxidase incubation, wash tissue sections with wash buffer.
2. Wipe slides removing excess buffer. Add enough drops of working Stable DAB/Plus solution to cover tissue sections.
3. Incubate for 5-10 minutes at room temperature. For optimal results, observe reaction under the microscope for signal development.
4. Once the desired signal to noise ratio is achieved, stop the reaction by washing slides in buffer.

V. Storage and Stability

Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.