

## DAB Chromogen/Substrate Kit (High Contrast)

**Description:** This product has been developed for applications that require high contrast between the chromogen and Hematoxylin counterstain. The resulting stain is a darker brown than standard DAB and somewhat more sensitive. ScyTek recommends using Hematoxylin for Automation (catalog # HAQ500) for optimal contrast. 3,3'-Diaminobenzidine (DAB) is a widely used chromogen for immunohistochemical staining and immunoblotting. When in the presence of peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol. This product is available in a two component form consisting of a liquid, refrigerator stable DAB Chromogen and DAB Substrate (High Contrast). The standard working dilution is 50ul (0.9mg) of DAB Chromogen per 1ml of DAB Substrate (High Contrast), although the ratio can be adjusted as desired. The use of liquid components reduces some risks associated with handling powders (ie. dust inhalation), and eliminates waste which often results from using tablets that require a predetermined final volume. Once the two components are combined, the reagent can be used for up to six hours, making it ideal for automated stainers.

**Contents:**

**Volume**

3ml DAB Chromogen, 1 vial  
5ml DAB Substrate (High Contrast), x11 vials

**Storage:** Store at 2-8°C. Each component is stable for 18 months from the date of manufacture.

**Procedure:**

1. Combine 5 drops of DAB Chromogen to DAB Substrate (High Contrast).
2. Apply mixture to tissue section.
3. Incubate tissue section for 5 minutes.
4. Rinse tissue with DI water.
5. Apply mixture and incubate for another 5 minutes.
4. Rinse tissue, counterstain, dehydrate, clear in xylene or xylene substitute and coverslip.

**Precautions:** DAB Chromogen: Contains Diaminobenzidine in buffer. DAB is a suspected carcinogen. Do not pipet by mouth. Avoid contact with skin and eyes. Reagent is acidic and can cause burns if skin contact occurs. Handle with care and dispose of according to regulations.  
DAB Substrate: Contains Hydrogen Peroxide in buffer. Avoid contact with skin and eyes.