

**DAB Chromogen & Substrate Kit: DR0253-100ML, DR0253-200ML**

**Intended Use:** For Research Use Only. This DAB kit is intended for use in peroxidase-based immunohistochemistry (IHC) staining protocols on formalin-fixed, paraffin embedded tissue (FFPE) sections.

**Description:** The chromogen is the final step in the detection portion of the IHC process; it enables the antibody-antigen complex to be viewed under the light microscope. 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. DAB acts as an electron donor in the presence of enzyme HRP Polymer. As a result, DAB gets reduced and the color change occurs. This kit includes two components consisting of a liquid, refrigerator stable DAB Chromogen and DAB Substrate. It is designed to provide excellent contrast between the chromogen and hematoxylin counterstain. The resulting stain is a dark brown and somewhat more sensitive than standard DAB. 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.

**Package:**

Description	Catalog No.	Size
DAB Chromogen & Substrate Kit	DR0253-100ML	100ml
DAB Chromogen & Substrate Kit	DR0253-200ML	200ml

**Reagents Supplied:** One bottle of DAB Substrate Buffer 100ml, or 200ml  
One bottle of DAB Chromogen 5ml, or 10ml

**Storage and Stability:** Store at 2°- 8°C. Do not freeze. All performance claims are void after the kit expiration date.

**Materials Required but Not Supplied:**

Xylene, graded EtOH, antigen retrieval solutions, peroxidase block, protein block, negative control reagents, primary antibody, conjugated secondary antibody, IHC wash buffer, hematoxylin, mounting medium, graduated cylinder, microscope slides, drying oven, positive and negative controls, slide rack, water bath, microwave or pressure cooker.

**Recommended Procedure:**

1. Before using, mix DAB Chromogen by repeated inversion.
2. Use a plastic test tube or other container to mix DAB Chromogen and Substrate. Pipette 50-80ul DAB Chromogen to every 1ml DAB Substrate and mix. The mixed working solution may be used for up to 6 hours. Note: 50µl /ml produces optimal staining quality, 80µl/ml produces maximum sensitivity.
3. Dip slide one time in DI/Distilled water immediately prior to application of DAB.
4. Apply working solution to tissue section and incubate tissue section for 5 minutes at room temperature.
5. Rinse slide using DI/Distilled water.
6. Apply working solution and incubate for another 5 minutes at room temperature. OPTIONAL.
7. Rinse slide in two changes of DI/Distilled water.
8. Counterstain as desired.
9. Dehydrate through graded alcohol, clear and coverslip in mounting medium.

**Quality Control:**

Refer NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on Tissue Controls.

**Limitations:**

Histological applications. Do not use if reagents become cloudy. Do not use past expiration date. Use caution when handling reagents. Non-Sterile.

The protocols involve multiple factors and steps that require trained lab professionals to operate. It is the responsibility of the investigator to determine optimal conditions.

**Warnings and Precautions:**

1. This product is for research use only by professionals only.
  2. Do not use after expiration date printed on product labels. The user must validate any storage conditions other than those specified in the package insert.
  3. Do not add more than 50ul DAB Chromogen to every 1ml DAB Substrate. Adding more DAB Chromogen may reduce staining intensity.
  4. Bring all reagents, slides, and specimens to room temperature (18-24°C) prior to use.
  5. Cross contamination of reagents or samples may give false results.
  6. Avoid microbial contamination of reagents, as this could produce incorrect results.
  7. Avoid contact of reagents with eyes and mucous membranes. If reagents contact with sensitive areas, wash with copious amounts of water.
  8. Do not smoke, eat, or drink in areas where specimens or reagents are handled.
  9. Avoid splashing or generation of aerosols at all time.
  10. Reusable glassware must be washed and thoroughly rinsed free of detergent prior to use. All glassware must be clean and dry before use.
  11. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with germicidal soap and copious amounts of water.
  12. Refer to product SDS.
  13. Do not store or use in strong light as product is photosensitive.
  14. Return product to 2-8°C when not in use as product is temperature sensitive.
  15. 3,3'-Diaminobenzidine tetrahydrochloride (a constituent of DAB Chromogen) is a potential carcinogen. Wear disposable gloves, handle with care, and do not breathe fumes.
  16. Do not freeze.
- Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

**Troubleshooting:**

1. Refer to reagent-specific protocol recommendation according to datasheet provided.
2. For further assistance, contact Medaysis at [Techsupport@medaysis.com](mailto:Techsupport@medaysis.com) or +1 510-509-3153.