

# DAB Substrate Kit

For In Vitro Diagnostic Use (IVD)  
Instructions for use

## INTENDED USE

DAB Substrate Kit is intended for use in peroxidase-based immunohistochemistry (IHC) staining protocols. The reagent is designated to detect target antigens in formalin-fixed, paraffin-embedded tissue sections when used in conjunction with antibodies and appropriate chromogen in the IHC staining process.

## SUMMARY AND EXPLANATION

When in the presence of HRP Polymer, DAB produces a brown precipitate that can be readily visualized using light microscopy.

## PRINCIPLES AND PROCEDURES

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. DAB acts as an electron donor in the presence of enzyme HRP Polymer. As a result, DAB gets reduced and the color change occurs.

## MATERIALS AND METHODS

### Reagents Supplied As:

Kit Cat. No.	Reagent Cat. No.	Contents	Vol. (ml)
957D-20 50 ml kit	957D-21	DAB Chromogen	3ml
	957D-22	DAB Buffer	50ml
957D-30 200 ml kit	957D-31	DAB Chromogen	12ml
	957D-32	DAB Buffer	200ml
957D-40 500ml ml kit	957D-41	DAB Chromogen	30ml
	957D-42	DAB Buffer	500ml
957D-50 15ml ml kit	957D-51	DAB Chromogen	1ml
	957D-52	DAB Buffer	15ml
957D-60 100ml ml kit	957D-61	DAB Chromogen	6ml
	957D-62	DAB Buffer	100ml

## Materials and Reagents Needed But Not Provided

- |  |                                |
|--|--------------------------------|
| 1. TBS or PBS wash buffer*                 | 8. Slide rack*                 |
| 2. Volumetric flask/graduated cylinder     | 9. Staining dishes*            |
| 3. Microscope slides, positively charged   | 10. Pressure cooker*           |
| 4. Drying oven                             | 11. Pretreatment reagents*     |
| 5. Positive and negative controls          | 12. Proteolytic enzyme         |
| 6. Clearing agent (xylene, Clearene, etc.) | 13. Avidin-Biotin block*       |
| 7. Ethanol or reagent alcohol              | 14. Peroxide block             |
|  | 15. Negative control reagents* |
|  | 16. Hematoxylin*               |
|  | 17. Mounting medium            |

\*See Cell Marque Catalog for product numbers. Some of the reagents listed are based on specific applications and detection system used.

## Storage and Stability

Store at 2-8°C up to a maximum of 24 month(s) from the date of manufacture (see product label for expiration date). Keep protected from light.

## Reagent Preparation

- Before using, mix DAB Chromogen by repeated inversion.
- Using a plastic test tube or other container, add recommended number drops of DAB Chromogen to desired volume of DAB Buffer Substrate and mix.
- After combining the two components, the working solution can be used for up to 5 days, if refrigerated.

Component	1 ml	5 ml
DAB Chromogen	1 drop	5 drops
DAB Buffer Substrate	1 ml	5 ml

## Recommended Protocol Instructions

- Cover tissue specimen completely with working solution of liquid DAB
- Incubate at room temperature for 5 minutes.
- Rinse slides in distilled water and counterstain.
- Dehydrate and coverslip with a permanent mounting medium.

## Protocol Notes

N/A

**INTERPRETATION OF RESULTS**

The Cell Marque DAB Substrate Kit causes a brown reaction product to precipitate at the antigen sites localized by the primary antibody. A qualified pathologist must evaluate controls and qualify the stained product before interpreting results.

**QUALITY CONTROL PROCEDURES**

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

**WARNINGS AND PRECAUTIONS**

1. This product is for *in vitro* diagnostic use 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. Bring all reagents, slides, and specimens to room temperature (18-24°C) prior to use.
4. Cross contamination of reagents or samples may give false results.
5. Avoid microbial contamination of reagents, as this could produce incorrect results.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Do not smoke, eat, or drink in areas where specimens or reagents are handled.
8. Avoid splashing or generation of aerosols at all times.
9. Reusable glassware must be washed and thoroughly rinsed free of detergent prior to use. All glassware must be clean and dry before use.
10. 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.
11. Refer to product SDS
12. Do not store or use in strong light as product is photosensitive.
13. Return product to 2-8°C when not in use as product is temperature sensitive.
14. 3,3'-Diaminobenzidine tetrahydrochloride (a constituent of DAB Chromogen) is a potential carcinogen. Wear disposable gloves, handle with care, and do not breathe fumes. Dispose of according to local regulations. See SDS for additional information.
15. Do not freeze.

**LIMITATIONS**

Immunohistochemistry is a multiple step diagnostic process that requires specialized training and selection of appropriate reagents and controls. The protocols for a specific application can vary. It is the responsibility of the end user to determine optimal conditions.

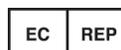
**TROUBLESHOOTING**

Refer to reagent-specific protocol recommendation according to data sheet provided.

For further help, feel free to contact Cell Marque's Technical Support at +1-800-665-7284.

**REFERENCES**

1. NCCLS Quality Assurance for Immunocytochemistry approved guideline, December 1999 MM4-A Vol. 19 No.26 for more information on tissue controls.

**DISCLAIMERS** [www.cellmarque.com](http://www.cellmarque.com)

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