PolyDetector DAB HRP Brown Substrate - Chromogen





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Intended Use

For In Vitro Diagnostic Use.

Summary and Explanation

The PolyDetector DAB HRP Brown Substrate-Chromogen is suitable for use in HRP Detection systems and allows for the demonstration of tissue antigens or nucleic acids in formalin-fixed paraffin-embedded tissues, frozen sections, cytosmears, and cell preparations. The substrate chromogen is the final step in the detection protocol and enables the antibody antigen complex or nucleic acid-probe complex to be viewed under the light microscope. DAB acts as an electron donor in the presence of the enzyme horseradish peroxidase (HRP). DAB gets oxidized and produces a brown color at the site of the target antigen or nucleic acid.

DAB forms a permanent stain and provides a record of the results when coverslipped with an organic based resin, such as PermaMounter (BSB 0094-BSB 0097) or biodegradable alternative, such as XyGreen PermaMounter (BSB 0169–BSB 0174) or water-based mounting media such as AquaMounter (BSB 0090-BSB 0093).

Presentation

DAB (3'3'-diaminobenzidine) is a chromogen (color forming molecule) that develops into a brown precipitate insoluble in alcohol and organic solvents.

Catalog No.	Buffer - Substrate	Chromogen	Catalog No.	Buffer - Substrate	Chromogen	Catalog No.	Buffer - Substrate	Chromogen
BSB 0015	15 mL	1 mL	BSB 0018B	1000 mL	50 mL	BSB 0019	1000 mL	-
BSB 0016	50 mL	3 mL	BSB 0019A	-	100 mL	BSB 0019D	500 mL	-
BSB 0017	100 mL	6 mL	BSB 0019B	-	50 mL	BSB 0019E	200 mL	-
BSB 0018	200 mL	12 mL	BSB 0019C	-	12 mL	BSB 0019F	100 mL	-
BSB 0018A	500 mL	25 mL	BSB 0019G	-	6 mL		•	

Storage Store at 2-8°C

Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: This product can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pretreatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033). Tissue should remain hydrated using Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: This product can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Preparation of Working Solution

The DAB Chromogen is concentrated and needs to be diluted with the DAB substrate buffer solution (1 drop chromogen/1 ml buffer). Shake the DAB Chromogen before dispensing into the substrate-buffer solution. Prepare the working DAB Substrate Chromogen Solution right before use.

Working DAB Substrate Chromogen Required	1 mL	2 mL	3 mL
DAB Buffer	1 mL	2 mL	3 mL
DAB Chromogen	1 drop	2 drops	3 drops

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector HRP	PolyDetector HRP	PolyDetector Plus HRP	
Peroxidase/AP Blocker	5 min.	5 min.	5 min	
Primary Antibody	30-60 min.	30-60 min.	30-60 min.	
1st Step Detection	10 min. 30-45 min.		15 min.	
2nd Step Detection	10 min.	Not Applicable	15 min.	
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.	
Counterstain / Coverslip	Varies	Varies	Varies	

Mounting Protocol

a. Alcohol/Xylene Protocol

- 1. After the histological, immunohistochemical or *in situ* hybridization staining procedure is completed, rinse slides in deionized water.
- 2. Dip the slides in alcohol 30%, 70%, and 100% for 1-2 minutes, then dip for 1-2 minutes in 3 xylenes.

3. Add an organic Permanent Mounting medium such as XyGreen PermaMounter (BSB 0169-0174), PermaMounter (BSB 0094-0097) or similar permanent mounting media. 4. Apply cover slip and air dry before microscopic observation.

b. ChromoProtector Protocol

1. After the histological, immunohistochemical or *in situ* hybridization staining procedure is completed, rinse slides in deionized water. Do not incubate tissue or cell specimens in solvents such as alcohol, toluene, or xylene.

2. Using a coplin jar, or a staining dish with a rack, immerse slides with tissues in ChromoProtector or lay wet slides horizontally and apply sufficient drops of

ChromoProtector (BSB 0151 – BSB 0156) to completely cover the tissue. Carefully spread ChromoProtector if needed, but avoid contacting the tissue.

3. Incubate slides for ten minutes at 60 °C to allow ChromoProtector to penetrate tissues.

4. Remove excess ChromoProtector by placing slides vertically over an absorbent material and let excess drain off into absorbent material. Do not rinse slides.

- 5. Allow slides to COMPLETELY air dry.
 - NOTE: The ChromoProtector will protect tissue from drying artifacts during the air-drying process.

6. When slides are completely dried, they can be mounted using most standard mounting methods such as aqueous or permanent.

7. Permanent Mounting

- Do not dehydrate slide through alcohol and/or xylene prior to mounting.
- Permanent Mounting medium such as XyGreenPermaMounter (Cat # BSB 0169-0174), PermaMounter (Cat # BSB 0094-0097) or similar permanent mounting media can be added directly to the slide until the tissue or cell specimen is covered.
- If the Permanent Mounting medium does not spread evenly on the dry slide, the slide can be dipped in toluene or xylene for 1 2 seconds to aid spreading of the mounting medium.
- Use a minimum amount of mounting medium so that slides dry rapidly.
- Apply coverslip and air dry before microscopic observation.

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

Precautions

- 1. For professional users only. Results should be interpreted by a qualified medical professional.
- 2. Ensure proper handling procedures are used with reagent. Minimize microbial contamination of reagents.
- 3. Always wear personal protective equipment such as laboratory coat, goggles, and gloves when handling reagents.
- 4. Dispose of unused solution according to local and federal regulations.
- 5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
- 6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
- 7. For additional safety information refer to Safety Data Sheet for this product.

8. For complete recommendations for handling biological specimens please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

References

1. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole







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