POLYDETECTOR DAB BROWN SUBSTRATE-CHROMOGEN

Intended Use For In Vitro Diagnostic Use.

Summary And Explanation

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

Presentation

DAB (3'3'diaminobenzidine) is a chromogen (color forming molecule) that develops into a brown precipitate. DAB forms a permanent record of the results of the stain when coverslipped with an organic based resin, such as **Perma Mounter** (BSB 0094-BSB 0097) or **XyGreen PermaMounter** (BSB 0169 – BSB 0174).

Availability

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

Storage Store at 2-8°C

Stability Stable up to the expiration date listed on the label. Do not use this product after the expiration date listed on the

product label.

Protocol

Preparation of Working Solutions

To prepare a working **PolyDetector DAB Brown Substrate-Chromogen** solution, first shake the **DAB Chromogen** solution, then add and mix 1 drop of **Chromogen** to 1 mL of **DAB Buffer-Substrate** per 1 mL of DAB Substrate-Chromogen required.

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

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, immerse slides with tissues in **ChromoProtector or l**ay 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.
- Organic 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 Organic 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.

Immunohistochemical Protocol

Step	ImmunoDetector HRP	PolyDetector HRP	PolyDetector Plus HRP	
Peroxidase Blocker	5 minutes	5 minutes	5 minutes	
Primary Antibody	30-60 minutes	30-60 minutes	30-60 minutes	
Secondary Biotinylated Link	10 minutes	N/A	15 minutes	
HRP Label	10 minutes	30-45 minutes	15 minutes	
ImmunoDetector DAB Substrate Chromogen	5-10 minutes	5-10 minutes	5-10 minutes	
Counterstaining	Time varies	Time varies	Time varies	

Precautions

- 1. For professional users only. Results should be interpreted by a medical professional.
- 2. Ensure proper handling procedures are used with reagent. Always wear proper personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
- 3. Minimize microbial contamination of reagents.z
- 4. Dispose of unused solution according to local and federal regulations.
- 5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.
- 6. Avoid contact with eyes. Flush with large quantities of water if contact occurs.
- 7. 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" (1).

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							
		2.0 A.c.	Storage Temperature Limites de température Zulassiger temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
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