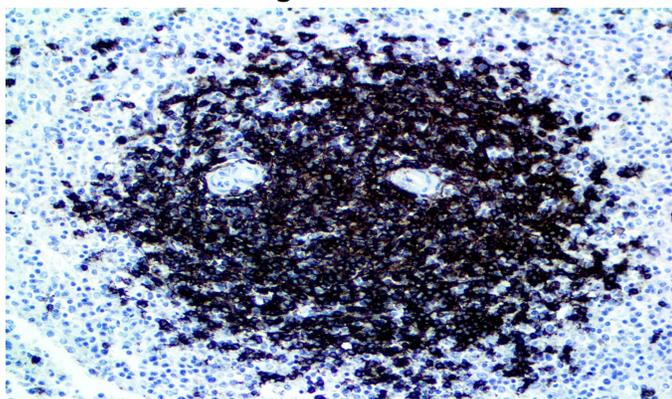


**PolyDetector HRP Black
Substrate-Chromogen**

RUO



Inset: IHC of CD20 on FFPE Spleen tissue with HRP Black Substrate-Chromogen

Intended Use

For Research Use Only.

Summary and Explanation

PolyDetector HRP Black Substrate-Chromogen is suitable for use with peroxidase detection systems and allows for the demonstration of cell 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 to be viewed under the light microscope. This occurs because HRP Black acts as an electron donor in the presence of the enzyme horseradish peroxidase; HRP Black gets oxidized and produces a gray/black color at the site of the target antigen or nucleic acid.

HRP Black forms a permanent record of the results of the stain when coverslipped with a ChromoProtector (BSB 0151-0156) and organic based mounting medium, such as PermaMunter (BSB 0094-BSB 0097) or XyGreen PermaMunter (BSB 0169 – BSB 0174). It can also be mounted with media such as AquaMunter (BSB 0090-BSB 0093) but the stain will change to brown within 24 hours. If Fast ChromoProtector (BSB 0327-15 to BSB 0327-1000) used or Fast Dip Xylene and OH rehydration protocol prior PermaMunter, the Black Signal will stay for about a 1 month.

Presentation

HRP Black is a chromogen (color forming molecule) that develops into a gray/black precipitate.

Catalog No.	Buffer-Substrate	Chromogen
BSB-0361-15	15 ml	0.5 ml
BSB-0361-50	50 ml	1.5 ml
BSB-0361-100	100 ml	3.0 ml
BSB-0361-200	200 ml	6.0 ml
BSB-0361-500	500 ml	14 ml
BSB-0361-1000	1000 ml	28 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.

Preparation of Working Solution.

To prepare a working PolyDetector HRP Black Substrate-Chromogen solution, first shake the HRP Black Chromogen solution, then add 1 drop of Chromogen to 1 mL of the HRP Black Buffer-Substrate for each 1 mL of Substrate-Chromogen solution required. Mix the two solutions well. Use this working solution within 5 minutes of preparation.

1 drop of HRP Black chromogen equals ~ 25 uL.

Working HRP Black Substrate Chromogen Required	1 ml	2 ml	3 ml
HRP Black Buffer	1 ml	2 ml	3 ml
HRP Black Chromogen	1 drop	2 drops	3 drops

Counterstaining

HRP Black can be counterstained with Nuclear Fast Red Counterstainer (BSB 0116- BSB 0121) for 2 min. or with Hematoxylin (BSB 0024 - BSB 0028) for 30- 60 seconds. For detailed counterstaining protocols refer to PIs 0121 or PI0028 respectively.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/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. Aqueous Mounting Protocol

1. After the histological, immunohistochemical or in situ hybridization staining procedure is completed, rinse slides with a TBST buffer (BSB 0042).
2. Apply 1-3 drops of an Aqueous Mounting medium such as AquaMounter (BSB 0090-0093) or similar mounting media.
3. Apply cover slip and air dry at room temperature before microscopic observation.

Ideal for immediate observation but the stain will turn brown within 24 hours.

b. ChromoProtector Protocol for permanent record

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. This product contains <0.1% sodium azide (NaN₃) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. Do not ingest reagents. If the reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to the Safety Data Sheet for this product.
9. 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

 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
 Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	 Expiration Date Utiliser jusque Verwendbar bis	 Lot Number Code du lot Chargenbezeichnung