Bio SB
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PolyDetector HRP Mulberry Substrate-Chromogen

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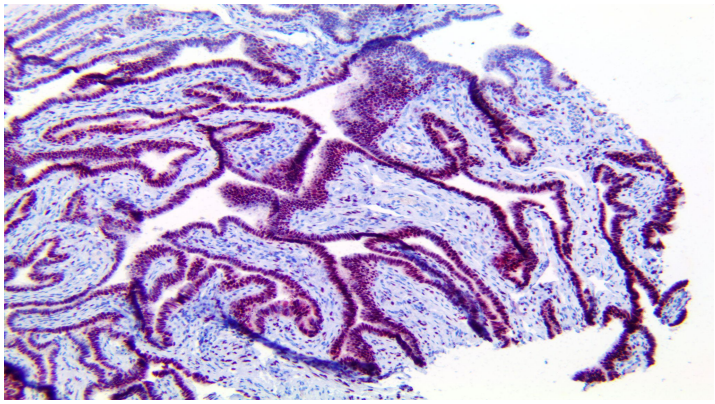

Inset: IHC of PR on a FFPE Fallopian Tube tissue using HRP Mulberry Substrate-Chromogen.

Intended Use
For Research Use Only.

Summary and Explanation
PolyDetector HRP Mulberry 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 Mulberry acts as an electron donor in the presence of the enzyme horseradish peroxidase; HRP Mulberry gets oxidized and produces a dark purple color tinted with red at the site of the target antigen or nucleic acid.

HRP Mulberry forms a permanent record of the stain results when coverslipped with a ChromoProtector (BSB 0151-0156) and organic based mounting medium, such as PermaMounter (BSB 0094-BSB 0097) or XyGreen PermaMounter (BSB 0169 – BSB 0174). It can also be mounted with media such as AquaMounter (BSB 0090-BSB 0093) but the stain will fade and crystalize within 24 hours.

Presentation
HRP Mulberry is a chromogen (color forming molecule) that develops into a dark purple color tinted with red precipitate.

Catalog No.	*Buffer-Substrate*	*Chromogen*
BSB-0362-15	15 ml	1.5 ml
BSB-0362-50	50 ml	4.5 ml
BSB-0362-100	100 ml	8.5 ml
BSB-0362-200	200 ml	16.5 ml
BSB-0362-500	500 ml	42 ml
BSB-0362-1000	1000 ml	83 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.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector HRP	PolyDetector HRP	PolyDetector Plus HRP
Peroxidase 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.	N/A	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Preparation of Working Solution.
To prepare a working PolyDetector HRP Mulberry Substrate-Chromogen solution add 3 drops of Chromogen to 1 mL of the HRP Mulberry Buffer-Substrate. Mix the two solutions well. Let it settle for 1 min. Use this working solution within 5 minutes of preparation.

1 drop of HRP Mulberry chromogen equals ~ 25 ul.

Working HRP Mulberry Substrate Chromogen Required	1 ml	2 ml	3 ml
HRP Mulberry Buffer	1 ml	2 ml	3 ml
HRP Mulberry Chromogen	3 drops	6 drops	9 drops

Counterstaining
HRP Mulberry can be counterstained for 30-60 seconds with Hematoxylin (BSB 0024 - BSB 0028). For detailed counterstaining protocols refer to PI 0028.

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.

Perfect for immediate observation but the signal will fade and crystalize 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.

Ensure proper handling procedures are used with this reagent.

2. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
3. Dispose of unused solution with copious amounts of water.
4. Do not ingest reagents. If the reagent is ingested, seek medical advice immediately.
5. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
6. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
7. For additional safety information refer to the 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

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

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