



For In Vitro Diagnostic Use

Mouse/Rabbit PolyDetector Plus DAB HRP Brown Detection System

Intended Use

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Summary and Explanation

The Mouse/Rabbit PolyDetector Plus HRP/DAB System is a 3-step polymeric detection system that allows for the demonstration of antigens in paraffin-embedded tissue, cryostat sections, blood smears, cytospins, and cell preparations. The PolyDetector kits have been developed using a proprietary tandem hyperlabeling technology used to directly label immunoglobulins with enzymes. This ensures consistent and reproducible immunostaining for all types of nuclear, cytoplasmic and membranous antigens, in different types of tissues.

The Mouse/Rabbit PolyDetector Plus HRP/DAB Detection System is suitable for use with mouse IgG and IgM and rabbit primary antibodies, both monoclonal and polyclonal. The Mouse/Rabbit PolyDetector Plus HRP/DAB Detection System kits are optimized for use with Bio SB primary antibodies; however, they are universal kits and therefore should work equally well with prediluted and concentrated antibodies from different vendors, as long as they are optimized.

Availability	
Catalog Number	Volume
BSB 0257S	5 mL Each
BSB 0257	15 mL Each
BSB 0259	50 mL Each
BSB 0261	100 mL Each
BSB 0263	200 mL Each
BSB 0265	1000 mL Each
BSB 0267	15 mL Plus Link & HRP Label
BSB 0268	50 mL Plus Link & HRP Label
BSB 0269	100 mL Plus Link & HRP Label
BSB 0270	200 mL Plus Link & HRP Label
BSB 0271	1000 mL Plus Link & HRP Label

Presentation

The **Mouse/Rabbit PolyDetector Plus DAB HRP Brown Detection System** contains a Peroxidase Blocker solution, a PolyDetector Plus Link Solution, an Anti-Mouse/Rabbit Horseradish Peroxidase solution, a DAB Buffer, and a DAB Chromogen solution. All the components are buffered with stabilizers and an anti-microbial.

Storage: Store at 2°C - 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

The **PolyDetector Peroxidase Blocker**, **PolyDetector Plus Link** and **Anti-Mouse/Rabbit Horseradish Peroxidase Label** are ready-to-use working solutions and require no further preparation. The **DAB Chromogen** is concentrated and needs to be diluted and mixed into the **DAB Buffer** to produce the working DAB substrate-chromogen solution. For each 1 mL of working DAB substrate-chromogen solution required for the experiment, 1 drop of **DAB Chromogen** should be added and mixed into 1 mL of **DAB Buffer**.

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 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.
- **Organic Permanent Mounting** medium such as **XyGreen PermaMounter** (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.

Recommended Immunohistochemical Protocol

1. Cut and mount 3-4 micron formalin-fixed paraffin-embedded tissues on positive charged slides.
2. Air dry for 2 hours at 58°C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as **ImmunoDNA Retriever with Citrate** (BSB 0020-BSB 0023) or **EDTA** (BSB 0030-BSB 0033).
5. Wash with 5 changes of IHC Wash buffer.
6. Place slides in **PolyDetector Peroxidase Blocker** for 5 min.
7. Wash with 3 changes of IHC wash buffer.
8. Cover tissue with the **Primary Antibody** for 30-60 minutes or follow manufacturer’s recommended protocol. If using concentrated antibodies, we suggest using our **ImmunoDetector Protein Blocker/Antibody Diluent** to dilute antibodies.
9. Wash with 3 changes of IHC wash buffer.
10. Cover tissue with **PolyDetector Plus Link**, incubate for 15 min.
11. Wash with 3 changes of IHC wash buffer.
12. Cover tissue with **PolyDetector HRP Label**, incubate for 15 min.
13. Wash with 3 changes of IHC wash buffer.
14. Prepare DAB by adding one drop of PolyDetector DAB Chromogen per mL of PolyDetector DAB Buffer and mix.
15. Cover tissue with prepared DAB substrate-chromogen solution, incubate for 5-10 min.
16. Rinse with 3 changes of IHC wash buffer
17. Counterstain and then dehydrate.
18. Coverslip.

Abbreviated Immunohistochemical Protocol	
Step	PolyDetector Plus HRP
Peroxidase Blocker	5 minutes
Primary Antibody	30 - 60 minutes
Plus Link	15 minutes
HRP Label	15 minutes
DAB Substrate-Chromogen	5-10 minutes
Counterstaining	Time varies with counterstain

Precautions

1. For professional users only. Results should 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. This product contains components with sodium azide (NaN₃), a toxic chemical. At product concentrations it is not classified as hazardous due to its low concentration. Sodium azide may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build up.
4. Minimize microbial contamination of reagents.
5. Dispose of unused solution according to local and federal regulations.
6. Do not ingest reagent. If reagent ingested, seek medical advice immediately.
7. Avoid contact with eyes. Flush with large quantities of water if contact occurs.
8. Follow safety precautions for the heating device (TintoRetriever Pressure Cooker or similar).
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” (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			
	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller
	 Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	 Expiration Date Utiliser jusque Verwendbar bis	 Catalog Number Référence du catalogue Bestellnummer
			 Lot Number Code du lot Chargenbezeichnung