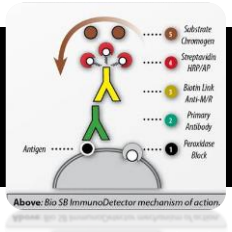
Bio SB
BIO SCIENCE FOR THE WORLD



Above: Bio SB ImmunoDetector mechanism of action.

IVD
For In Vitro Diagnostic Use

Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System

Intended Use
For In Vitro Diagnostic Use.

Summary and Explanation
The **Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System** is a Biotin-Streptavidin-Horseradish Peroxidase Detection System that allows for the demonstration of antigens in paraffin-embedded tissue, cryostat sections, cytosmears, and cell preparations. The increased sensitivity of the **Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System** allows for rapid staining procedures without compromises in the quality of stains.

The **Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System** is suitable for use with mouse or rabbit primary antibodies. **The Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System** kits are optimized for use with Bio SB primary antibodies; however, they are universal kits and therefore work equally well with prediluted and concentrated antibodies from different vendors.

Presentation
The **Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System** contains a Peroxidase Blocker solution, a Biotinylated Anti-Mouse and Anti-Rabbit immunoglobulin solution, a Streptavidin conjugated to Horseradish Peroxidase Peroxidase solution, a DAB Buffer, and a DAB Chromogen solution. All the components are buffered with stabilizers and an anti-microbial.

Availability	
Catalog No.	**Volume**
BSB 0001S	5 mL Each
BSB 0001	15 mL Each
BSB 0001L	15 mL Link Only
BSB 0001H	15 mL Label Only
BSB 0001LH	15 mL Link and Label
BSB 0003	50 mL Each
BSB 0003L	50 mL Link Only
BSB 0003H	50 mL Label Only
BSB 0003LH	50 mL Link and Label
BSB 0005	100 mL Each
BSB 0005L	100 mL Link Only
BSB 0005H	100 mL Label Only
BSB 0005LH	100 mL Link and Label
BSB 0007	200 mL Each
BSB 0007L	200 mL Link Only
BSB 0007H	200 mL Label Only
BSB 0007LH	200 mL Link and Label
BSB 0009	1000 mL Each
BSB 0009L	1000 mL Link Only
BSB 0009H	1000 mL Label Only
BSB 0009LH	1000 mL Link and Label

Storage: 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 **ImmunoDetector Peroxidase Blocker**, **Anti-Mouse/Rabbit Biotinylated Link,** and **Streptavidin conjugated HRP 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 60ºC 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 **ImmunoDetector Peroxidase Blocker** for 5 min.
7. Wash with 3 changes of IHC wash buffer.
8. Cover tissue with the **Primary Antibody** following 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 **ImmunoDetector Biotin Link,** incubate for 10 min.
11. Rinse with 3 changes of IHC wash buffer.
12. Cover tissue with **ImmunoDetector HRP Label,** incubate for 10 min.
13. Rinse with 5 changes of DI water.
14. Prepare DAB by adding one drop of **ImmunoDetector DAB Chromogen** per mL of **ImmunoDetector DAB Buffer** and mix.
15. Cover Tissue with prepared DAB substrate-chromogen solution, incubate for 5 min.
15. Rinse with 5 changes of DI water.
16. Counterstain and then dehydrate.
17. Coverslip.

Abbreviated Immunohistochemical Protocol	
Step	**ImmunoDetector HRP**
Peroxidase Blocker	5 minutes
Primary Antibody	30 – 60 minutes
Biotin Link	10 minutes
HRP Label	10 minutes
DAB Substrate-Chromogen	5 – 10 minutes
Counterstaining	Time varies with counterstain

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. This product contains components with sodium azide (NaN_3), 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 Symbols

	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
IVD In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	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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