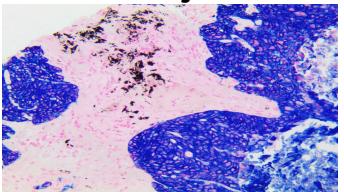




PolyDetector HRP Blue Plus Substrate - Chromogen





Inset: IHC of CK AE1 & AE3 on a FFPE Lung Squamous Cell Carcinoma Tissue

Intended Use

For Research Use Only.

Summary and Explanation

The PolyDetector HRP Blue Plus Substrate-Chromogen Kit is suitable for use with peroxidase detection systems, which allow 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 of IHC; it enables the antibody-antigen or nucleic acid complex to be viewed under a light microscope. This occurs because HRP Blue Plus acts as an electron donor in the presence of the enzyme horseradish peroxidase; HRP Blue Plus gets oxidized and produces a vibrant blue color at the site of the target antigen or nucleic acid. This substrate-chromogen is particularly useful in tissues where there is endogenous melanin or with multiplex IHC or CISH.

HRP Blue Plus is suitable for aqueous mounting such as AquaMounter (BSB 0090-BSB 0093). HRP Blue Plus is soluble in xylenes and therefore cannot be mounted with xylene protocol unless when using the Bio SB Fast ChromoProtector (BSB 0327-15 - BSB 0327-1000), then an organic Permanent Mounting medium such as PermaMounter (BSB 0097).

Presentation

The PolyDetector HRP Blue Plus Substrate-Chromogen Kit contains a liquid HRP Blue Plus Chromogen and an HRP Blue Plus Buffer Substrate containing a buffer, hydrogen peroxide as a substrate, and a non-azide anti-microbial.

Catalog No.	Buffer-Substrate	Chromogen	
BSB-0363-15	15 mL	1 mL	
BSB-0363-50	50 mL	3.5 mL	
BSB-0363-100	100 mL	7 mL	
BSB-0363-200	200 mL	14 mL	
BSB-0363-500	500 mL	35 mL	
BSB-0363-1000	1000 mL	70 mL	

Storage Store at 2-8°C

Stability

This product is stable up to the expiration date on the product label.

Do not use the after expiration date listed on the package label. Temperature fluctuations should be avoided. Store appropriately when not in use. Adhere to all local laws when disposing of this product.

Preparation of Working Solution

To prepare a working PolyDetector HRP Blue Plus Substrate-Chromogen solution, add and mix 2 drops of the HRP Blue Plus Chromogen per 1 mL of HRP Blue Plus Buffer-Substrate. Once mixed, use within 10 days when stored at 2 - 8 °C and in the dark.

1 drop of HRP Blue Plus Chromogen equals ~30 ul.

Working HRP Blue Plus Substrate Chromogen Required	0.5 mL	1 mL	1.5 mL
HRP Blue Plus Buffer-Substrate	0.5 mL	1 mL	1.5 mL
HRP Blue Plus Chromogen	1 drop	2 drops	3 drops

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 min.	5 min.	5 min.
Counterstain/Coverslip	Varies	Varies	Varies

Counterstaining

HRP Blue Plus can be counterstained with Nuclear Fast Red Counterstainer (BSB 0116- BSB 0121) for 1-10 minutes, with Pink Hematoxylin (BSB 0360-15 - BSB 0360-1000) for 30-60 seconds or with Hematoxylin (BSB 0024 - BSB 0028) for 30-60 seconds. For detailed counterstaining protocols, refer to PIs 0121, 0360 or 0028, respectively.

Mounting Protocols

a. Aqueous Mounting Protocol

- 1. After the histological, immunohistochemical or in situ hybridization staining procedure is completed, rinse slides with 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.

The signal is preserved for at least 2 months using this Aqueous Mounting method.

b. Fast ChromoProtector Mounting Protocol with CapGap

- 1. After the histological, immunohistochemical or in situ hybridization staining procedure is completed, rinse slides with TBST buffer (BSB 0042).
- 2. Draw the Fast ChromoProtector (BSB 0327-15 BSB 0327-1000) up into the capillary gap, completely covering the tissue.
- 3. Allow it to sit for 30 seconds to 1 minute, then separate the slides and allow the Fast ChromoProtector to drain off.
- 4. Let slides dry completely and mount with an organic Permanent Mounting medium such as PermaMounter (BSB 0097).

The signal is preserved for at least 8 months using this Permanent Mounting method.

c. Fast ChromoProtector Mounting Protocol on single slides

- 1. After the histological, immunohistochemical or in situ hybridization staining procedure is completed, rinse slides with TBST buffer (BSB 0042) and let all excess buffer drain off by tilting slides.
- 2. Apply 1-3 drops of FastChromoProtector (BSB 0327-15 BSB 0327-1000) directly on the tissue and allow it to sit for 30 seconds. Let it drain off completely by tilting slides.
- 3. Let slides dry completely and mount with an organic Permanent Mounting medium such as PermaMounter (BSB 0097).

The signal is preserved for at least 8 months using this Permanent Mounting method.

Product Limitations

Due to inherent variability present in IHC 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. Ensure proper handling procedures are used with this reagent. Minimize microbial contamination of reagents.
- 3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
- 4. Dispose of unused solution according to local and federal regulations.
- 5. Do not ingest reagent. If 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 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 WorkPractices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

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