



*Inset: IHC of CK5 on Skin Tissue*

### Intended Use

For In Vitro Diagnostic Use.

### Summary and Explanation

The Mouse/Rabbit ImmunoDetector Alkaline Phosphatase/ ALK Scarlet Detection System is a Biotin-Streptavidin-Alkaline Phosphatase Detection System that allows for the demonstration of antigens in formalin-fixed paraffin-embedded tissue, cryostat sections, cytosmeears, and cell preparations. The increased sensitivity of ImmunoDetector AP/ALK Scarlet Detection System allows for rapid staining procedures without compromises in the quality of stains.

The ImmunoDetector AP Blocker is used to block the endogenous Alkaline Phosphatase enzymes that naturally occur in cells and tissue sections without affecting antigens or nucleic acids. The Mouse/Rabbit ImmunoDetector Alkaline Phosphatase Detection System is suitable for use with mouse (IgG and IgM) and rabbit primary monoclonal and polyclonal antibodies. The substrate chromogen is the final step in the detection portion; it enables the antibody-antigen-enzyme complex to be viewed under the light microscope. This occurs when Alk Scarlet, in the presence of Alkaline Phosphatase, gets deposited at the site of the target antigen, producing a scarlet color that is partially soluble in organic solvents, and therefore care should be taken when mounting with permanent mounting media (please refer to the recommended permanent mounting protocol on reverse). The ImmunoDetector AP/ALK Scarlet Detection System kits are universal kits and therefore work equally well with prediluted and concentrated antibodies from different vendors.

### Presentation

The ImmunoDetector Alkaline Phosphatase Detection System contains an Alkaline Phosphatase Blocker solution, a Link of Biotinylated Anti-Mouse and Anti-Rabbit immunoglobulin solution, a Streptavidin conjugated to Alkaline Phosphatase solution, an ALK Scarlet chromogen solution, and an ALK Scarlet Buffer-Substrate solution. All the components are buffered with stabilizers and a non-azide anti-microbial agent.

Kit	Link	Label	Link & Label	Volume
BSB-0350-15	BSB 0001L	BSB 0082A	BSB 0082LA	15 mL
BSB-0350-50	BSB 0003L	BSB 0083A	BSB 0083LA	50 mL
BSB-0350-100	BSB 0005L	BSB 0084A	BSB 0084LA	100 mL
BSB-0350-200	BSB 0007L	BSB 0085A	BSB 0085LA	200 mL
BSB-0350-1000	BSB 0009L	BSB 0086A	BSB 0086LA	1000 mL

**Storage** Store at 2-8°C

### Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN<sub>3</sub>) 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 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).

### Stability

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

Do not use after expiration date listed on the 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

The AP Blocker, ImmunoDetector Link Anti-Mouse/Rabbit, and AP Label are ready-to-use working solutions and require no further preparation. To prepare a working ALK Scarlet Substrate-Chromogen solution, first shake the ALK Scarlet well Chromogen solution, then add 1 drop of Chromogen to 1 mL of Substrate-Chromogen solution. Mix the two solutions well. Use this working solution within 5-10 minutes of preparation.

### Specimen Preparation

**Paraffin sections:** The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033), or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

**Frozen sections and cell preparations:** The antibody can be used on acetone-fixed frozen sections and acetone-fixed cell preparations.

## IHC Protocol

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate, and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

### a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

### b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

### c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

## Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP
Peroxidase/AP Blocker	5 min.
Primary Antibody	30-60 min.
1st Step Detection	10 min.
2nd Step Detection	10 min.
Substrate- Chromogen	5-10 min.
Counterstain /Coverslip	Varies



## Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

## 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.

## 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.  
<https://www.cdc.gov/mmwr/pdf/other/su6101.pdf>

## Symbol Key / Légende des symboles/Erläuterung der Symbole

<b>EC</b> <b>REP</b>	QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	<b>REF</b>	Catalog Number Référence du catalogue Bestellnummer
<b>IVD</b>	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	<b>LOT</b>	Lot Number Code du lot Chargenbezeichnung