

*Inset: IHC of CK AE1/AE3 with ALK Magenta on Squamous Cell Carcinoma tissue*

### Intended Use

For In Vitro Diagnostic Use.

### Presentation

PolyDetector ALK Magenta Substrate-Chromogen contains an ALK Magenta chromogen solution, an ALK Magenta Activator solution and an ALK Magenta Buffer-Substrate solution. All the components are buffered, contain stabilizers and a non-azide anti-microbial.

### Summary and Explanation

PolyDetector ALK Magenta Substrate-Chromogen is suitable for use in immunohistochemical, Immunocytochemical and in situ hybridization procedures using Alkaline Phosphatase Detection Systems. PolyDetector ALK Magenta Substrate-Chromogen allows for the demonstration of cell antigens or nucleic acids in paraffin-embedded tissues, cryostat sections, cytospins, and cell preparations.

The substrate-chromogen is the final step in the detection portion; it enables the antibody-antigen-enzyme (or nucleic acid-probe-enzyme) complex to be viewed under the light microscope. This occurs when ALK Magenta, in the presence of Alkaline Phosphatase, gets deposited at the site of the target antigen or nucleic acid, producing a magenta color that is partially soluble in organic solvents and therefore care should be taken when mounting with a fast permanent mounting protocol or it can be mounted with permanent medium when using the Bio SB ChromoProtector (BSB 0151 – BSB 0156).

| Catalog No. | Buffer-Substrate | Chromogen | Activator |
|-------------|------------------|-----------|-----------|
| BSB 0077    | 15 ml            | 1 mL      | 1 ml      |
| BSB 0078    | 50 ml            | 3 mL      | 3 ml      |
| BSB 0079    | 100 ml           | 6 mL      | 6 ml      |
| BSB 0080    | 200 ml           | 12 mL     | 12 ml     |
| BSB 0081    | 1000 ml          | 50 mL     | 50 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 the 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 ALK Magenta Substrate-Chromogen solution, first shake the ALK Magenta Chromogen solution, then add 1 drop of ALK Magenta Chromogen to 1 drop of ALK Magenta Activator and mix. After 10-20 seconds, add 1 ml of the ALK Magenta Buffer Substrate solution to the chromogen-activator and mix again. Use this working solution within 5-10 min of preparation.

| Working ALK Magenta Substrate Chromogen Required | 1 ml   | 2 ml    | 3 ml    |
|--|--------|---------|---------|
| ALK-Magenta Chromogen                            | 1 drop | 2 drops | 3 drops |
| ALK-Magenta Activator                            | 1 drop | 2 drops | 3 drops |
| ALK-Magenta Buffer-Substrate                     | 1 ml   | 2 ml    | 3 ml    |

### Recommended Immunohistochemical Protocol

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

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

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
3. Ensure proper handling procedures are used with reagent. Minimize microbial contamination of reagents.
2. Always wear personal protective equipment such as laboratory coats, goggles and gloves when handling reagents.
3. Dispose of unused solution with copious amounts of water.
4. Do not ingest reagent. If 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 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)

## Mounting Protocol

### a. Alcohol/Xylene Protocol (Fast)

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 3-5 seconds, then dip for 3-5 seconds in 3 xylenes.
3. Add an organic Permanent Mounting medium such as XyGreen PermaMunter (BSB 0169-0174), PermaMunter (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. 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. Alternatively, add the ChromoProtector solution to a coplin jar or staining dish and submerge the slides in the solution.
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. Add an organic Permanent Mounting medium such as XyGreen PermaMunter (BSB 0169-0174), PermaMunter (BSB 0094-0097) or similar permanent mounting media.
7. Apply cover slip and air dry before microscopic observation.

### c. Fast ChromoProtector Protocol

1. Apply a few drops of Fast ChromoProtector onto the tissue. Tilt the slide and let the excess drain off the slide. If using capillary gap, draw the Fast ChromoProtector into the gap and incubate for 1-2 min. Let the slides dry completely.
2. Apply an organic permanent mounting medium and cover slip.









## 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.

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

|   |  |   |   |  |   |   |   |
|---|--|---|---|--|---|---|---|
|  | QAdvis EAR AB<br>Ideon Science Park<br>Scheelevägen 17<br>SE-223 70 Lund, Sweden                         |  | Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich                           |  | Manufacturer<br>Fabricant<br>Hersteller               |  | Catalog Number<br>Référence du catalogue<br>Bestellnummer |
|  | In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro<br>In-Vitro-Diagnostikum |  | Read Instructions for Use<br>Consulter les instructions<br>d'utilisation<br>Gebrauchsanweisung beachten |  | Expiration Date<br>Utiliser jusqu'à<br>Verwendbar bis |  | Lot Number<br>Code du lot<br>Chargenbezeichnung           |