

## Polink-2 Plus HRP Anti- Goat AEC Detection Kit Super Sensitive to AEC Chromogen

(2-step Polymer-HRP detection system, biotin-free,)  
**Polymer Detection System with Super Sensitivity and Specificity**

Storage: 4-8°C

|             |                                  |                                       |
|-------------|----------------------------------|---------------------------------------|
| Catalog No. | <input type="checkbox"/> D45-110 | 110 ml (bulk, w/o chromogen)          |
|             | <input type="checkbox"/> D45-18  | 18 ml (with AEC, good for 180 slides) |
|             | <input type="checkbox"/> D45-6   | 6 ml (with AEC, good for 50 slides)   |

### Intended Use:

Polink-2 Plus HRP anti Goat AEC Detection Kit is the 3rd generation of polymer detection system. It uses Goat antibody specific enhancer to help amplify the polymer-enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. This kit is extremely sensitive to AEC chromogen. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of Polink-2 Plus detection system. Polink-2 Plus HRP anti Goat DAB Detection Kit does not cross react with bovine IgG. It is compatible with BSA containing diluent or blocking buffer. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. It can be used for manual stain or autostainer. Staining conditions need to be optimized by user. DAB chromogen is NOT recommended to use with this system.

Polink-2 Plus HRP AEC Detection System offers a wide choice for primary antibodies, including broad spectrum (for mouse and rabbit primary antibodies), mouse, rabbit, goat, and rat primary antibodies. Refer to **Related Product** section for details.

### Kit components:

| Catalog No. | Product Name                            | Reagent 1:<br>Goat Antibody Enhancer<br>(Ready-to-use) | Reagent 2:<br>Polymer HRP for Goat<br>(Ready-to-use) | Reagent3A, 3B, 3C:<br>3A: AEC Substrate (20x)<br>3B: AEC Chromogen (20x)<br>3C: Hydrogen Peroxide (20x) |
|-------------|---|--|--|---|
| D45-110     | Polink-2 Plus HRP Goat for AEC Bulk kit | 110ml  | 110ml  | Not included  |
| D45-18      | Polink-2 Plus HRP Goat AEC 18ml kit     | 18ml   | 18ml   | Reagent 3A: 2ml<br>Reagent 3B: 4ml<br>Reagent 3C: 2ml   |
| D45-6       | Polink-2 Plus HRP Goat AEC 6ml kit      | 6ml  | 6ml  | Reagent 3A: 1ml<br>Reagent 3B: 2ml<br>Reagent 3C: 1ml   |

### Recommended Protocol:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, user needs to supply appropriately fixed tissue and well prepared slides.
2. Tissue need to be adhered to the slide tightly to avoid tissue falling off.
3. Paraffin embedded section must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made into as thin monolayer as possible to obtain satisfactory results.
5. Investigator needs to optimize dilution and incubation times for primary antibodies.
6. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
7. Staining steps: DO NOT let specimen or tissue dry from this point on.

| Reagent   | Staining Procedure   | Incubation Time |
|---|--|-----------------|
| 1. PEROXIDASE BLOCKING REAGENT.<br>Supplied by user | a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H <sub>2</sub> O <sub>2</sub> solution) for 10 minutes.                         | 10 min.         |
|   | b. Rinse the slide using distilled water.  |                 |
| 2. HIER PRETREATMENT:                               | a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. Please check the data sheet of primary antibody |                 |
|   | b. Wash with PBS 3 times each for 2 minutes.   |                 |

|  |  |                      |
|--|--|----------------------|
| 3. PRIMARY ANTIBODY<br>Supplied by user  | a. Apply 2 drops (100 µL) or enough volume of PRIMARY ANTIBODY to cover the tissue section completely. Incubate in moist chamber for 30-60 min.  | 30-60 min.           |
|  | b. Rinse with PBS 3 times each for 2 minutes.  |                      |
| 4. Goat Antibody Enhancer (Ready-to-use).<br><b>Reagent 1</b>  | a. Apply 2 drops (100 µL) or enough volume of Goat Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30 min.<br><b>(We recommend incubating the antibody enhancer up to 30mins for best sensitivity)</b>   | 10-30 min.           |
|  | b. Rinse with PBS for 2 min., 3 times.   |                      |
| 5. POLYMER-HRP for Goat (Ready-to-use)<br><b>Reagent 2</b>   | a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for GOAT to cover each section. Incubate in moist chamber for 10-30 min.<br><b>(We recommend incubating the polymer up to 30mins for best sensitivity)</b>   | 10-30 min.           |
|  | b. Rinse with PBS 3 times each for 2 minutes.  |                      |
| 6. CHROMOGEN<br><b>Reagent 3A:</b> AEC Substrate (20x)<br><b>Reagent 3B:</b> AEC Chromogen (20x)<br><b>Reagent 3C:</b> Hydrogen Peroxide (20x) | a. Add 1 drop of Reagent <b>3A</b> into 1ml distilled water, mix well. Then add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent <b>3B</b> and 1 drop of Reagent <b>3C</b> in the diluted substrate buffer. Mix well. Protect from light and use within 1 hour.<br>b. Apply 2 drops (100 µL) or enough of mixture completely cover tissue. Incubate for about 5 min. Monitor the color development under microscope.<br>c. Rinse with tap water for 1-2 min.<br><b>AEC is alcohol soluble, do not dehydrate!</b> | 5 min.               |
| 7. HEMATOXYLIN<br>Supplied by user   | a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue completely and wait about 15-20 <b>seconds</b> .   | 15-20 <b>seconds</b> |
|  | b. Rinse well under tap water for 1-2 minutes.   |                      |
|  | c. Put slides in PBS until show blue color (about 30-60 <b>seconds</b> ).  |                      |
|  | d. Rinse well in distill or tap water  |                      |
| 8. Mounting medium:<br>Supplied by user  | Follow the manufacture data sheet procedure for mounting.<br>Recommended product:<br>1. GB-Mount: Cat. No. E01-18 (18ml), for alcohol soluble substrates (AEC, AP-Red, and AP-blue)<br>2. Simpo-Mount: Cat.No. E03-18 (18ml), or E03-100 (100ml), universal permanent mounting medium. Can be used with or without cover slip  | Refer to insert      |

#### Protocol Notes:

The fixation, tissue slide thickness, and primary antibody dilution and incubation time affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting the result.

#### Related Products:

| Product   | Catalog No.     | Size        | Product  | Catalog No.       | Size          |
|---|-----------------|-------------|--|-------------------|---------------|
| Polink-2 Plus HRP Mouse for AEC bulk kit (without chromogen)                | D38-110         | 110ml       | Polink-2 Plus HRP Rat-NM for AEC (No cross react to mouse ) Bulk kit (without chromogen) | D48-110           | 110ml         |
| Polink-2 Plus HRP Mouse AEC kit   | D38-18<br>D38-6 | 18ml<br>6ml | Polink-2 Plus HRP Rat-NM (No cross react to mouse) AEC kit                               | D48-18<br>D48-6   | 18ml<br>6ml   |
| Polink-2 Plus HRP Rabbit for AEC Bulk Kit (without chromogen)               | D40-110         | 110ml       | Polink-2 Plus HRP Mouse-NR (No cross react to Rat) Bulk kit (without chromogen)          | D59-110           | 110ml         |
| Polink-2 Plus HRP Rabbit AEC kit  | D40-18<br>D40-6 | 18ml<br>6ml | Polink-2 Plus HRP Mouse-NR (No cross react to Rat) AEC kit                               | D59-18<br>D59-6   | 18ml<br>6ml   |
| Polink-2 Plus HRP Broad for AEC Bulk Kit (Without chromogen. For Ms. & Rb.) | D42-110         | 110ml       | AEC Kit (20x concentrated)   | C01-12            | 12ml          |
| Polink-2 Plus HRP Broad AEC Kit (for mouse and Goat antibody)               | D42-18<br>D42-6 | 18ml<br>6ml | Simpo-mount  | E03-18<br>E03-100 | 18ml<br>100ml |

**Precautions:** Please wear gloves and take other necessary precautions.

**Remarks:** For research use only.