

## Polink-2 Plus HRP Human DAB Detection System for Immunohistochemistry

(2-step Polymer-HRP Detection System with Super Sensitivity and Specificity, Biotin-Free)

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|----------------|
| Storage: 4-8°C |
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|             |                          |         |  |
|-------------|--------------------------|---------|--|
| Catalog No. | <input type="checkbox"/> | D88-110 | 110mL (with DAB, good for 1100 slides)     |
|             | <input type="checkbox"/> | D88-18  | 18mL (with DAB, good for 180 slides)       |
|             | <input type="checkbox"/> | D88-6   | 6mL (with DAB, good for 50 slides)         |
|             |                          |         | <i>*Total volume of polymer Conjugates</i> |
|             |                          |         | <i>** if use 100µL per slide</i>           |

### Intended Use:

The **Polink-2 Plus HRP Human Kit** is designed to use with user supplied human antibody to detect human antigens on mouse tissue or cell samples. This kit has been tested in paraffin tissue. However, this kit can be used on frozen specimen and freshly prepared monolayer cell smears.

This kit is the 3rd generation of polymer detection system. Human antibody enhancer is used to help amplify the polymer-enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies. User may need to titer primary antibody due to super sensitivity of Polink-2 Plus detection system. The **Polink-2 Plus HRP Human Kit** is non-biotin system that avoids endogenous biotin non-specific binding, which is suitable for manual or autostaining. This kit is not used for screening human or humanized antibodies on human tissue.

Polink-2 Plus HRP 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:

| Component No.     | Content                      | 6mL Kit | 18mLKit | 110mL Kit |
|-------------------|------------------------------|---------|---------|-----------|
| <b>Reagent 1</b>  | Human Antibody Enhancer(RTU) | 6mL     | 18mL    | 110mL     |
| <b>Reagent 2</b>  | Polymer HRP for Human(RTU)   | 6mL     | 18mL    | 110mL     |
| <b>Reagent 3A</b> | DAB Substrate(RTU)           | 12mL    | 15mLx2  | NA        |
| <b>Reagent 3B</b> | DAB Chromogen(20x)           | 1.5mL   | 2mL     | NA        |

### 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 as much 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 (Min.) |
|---|---|------------------------|
| 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.<br>b. Rinse the slide with distilled water.  | 10                     |
| 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.<br>b. Wash with PBS containing 0.05% Tween-20 for 2 min, 3 times. | ~1h                    |
| 3. Pre-Block:<br>Supplied by user                   | Pre-Block is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.   |                        |

|  |   |                 |
|--|---|-----------------|
| 4. PRIMARY ANTIBODY<br>Supplied by user  | a. Apply 2 drops (100µL) or enough volume of PRIMARY ANTIBODY to cover the tissue section completely. Incubate in moist chamber for 30-60 min.<br>b. Rinse with PBS containing 0.05% Tween-20 for 2 min, 3 times.   | 30-60           |
| 5. <b>Reagent 1</b><br><b>Human Antibody Enhancer</b><br>(RTU)                                       | a. Apply 2 drops (100µL) or enough volume of <b>Reagent 1</b> Human Antibody Enhancer to cover each section. Incubate in moist chamber for 10 min.<br>b. Rinse with PBS containing 0.05% Tween-20 for 2 min, 3 times.   | 10              |
| 6. <b>Reagent 2</b><br><b>Polymer HRP for Human</b><br>(RTU)   | a. Apply 2 drops (100µL) or enough volume of <b>Reagent 2</b> Polymer HRP for Human to cover each section. Incubate in moist chamber for 10 min.<br>b. Rinse with PBS containing 0.05% Tween-20 for 2 min, 3 times.   | 10              |
| 7. CHROMOGEN<br><b>Reagent 3A: DAB</b><br>Substrate(RTU)<br><b>Reagent 3B: DAB</b><br>Chromogen(RTU) | a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of <b>Reagent 3B</b> into 1ml of <b>Reagent 3A</b> . Mix well. Protect from light and use within 5 hours.<br>b. Apply 2 drops (100µL) or enough DAB working solution to completely cover each section. Incubate for 5 min. Monitor the color development under microscope.<br>c. Rinse with tap water for 1-2 min. | 5               |
| 8. HEMATOXYLIN<br>Supplied by user   | a. Counterstain with 2 drops (100µL) or enough volume of Hematoxylin to cover tissue completely and incubate about 15-20 seconds.<br>b. Rinse well with running tap water for 1-2 minutes.<br>c. Put slides in PBS until show blue color (about 30-60 seconds).<br>d. Rinse well in distilled water or tap water.   | 15-20Sec        |
| 9. 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 AEC, AP-Red, and AP-blue.<br>2. O-Mount: Cat. No. E02-18 (18mL), for DAB<br>3. 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:

1. The fixation, tissue slide thickness, and primary antibody dilution and incubation time effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.
2. Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.

#### Related Products:

| Product   | Catalog No.     | Size        | Product  | Catalog No.     | Size           |
|---|-----------------|-------------|--|-----------------|----------------|
| Polink-2 Plus HRP Mouse Bulk Kit (without chromogen)                | D37-110         | 110mL       | Polink-2 Plus HRP Rat-NM (No cross react to mouse ) Bulk Kit (without chromogen) | D46-110         | 110mL          |
| Polink-2 Plus HRP Mouse with DAB Kit                                | D37-18<br>D37-6 | 18mL<br>6mL | Polink-2 Plus HRP Rat-NM (No cross react to mouse) with DAB Kit                  | D46-18<br>D46-6 | 18mL<br>6mL    |
| Polink-2 Plus HRP Goat Bulk Kit (without chromogen)                 | D43-110         | 110mL       | Polink-2 Plus HRP Mouse-NR (No cross react to Rat) Bulk Kit (without chromogen)  | D58-110         | 110mL          |
| Polink-2 Plus HRP Goat with DAB Kit                                 | D43-18<br>D43-6 | 18mL<br>6mL | Polink-2 Plus HRP Mouse-NR (No cross react to Rat) with DAB Kit                  | D58-18<br>D58-6 | 18mL<br>6mL    |
| Polink-2 Plus HRP Broad Bulk Kit (Without chromogen. For Ms. & Rb.) | D41-110         | 110mL       | DAB Kit (2-components)   | C09-12          | 12mL<br>+240mL |
| Polink-2 Plus HRP Broad DAB Kit (for mouse and rabbit antibody)     | D41-18<br>D41-6 | 18mL<br>6mL | O-Mount (Organic)  | E02-18          | 18mL           |

**Precautions:** DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

**Remarks:** For research use only.