



# Polink-2 Plus AP Mouse Detection System for Immunohistochemistry

(2-step Polymer-AP detection system, biotin-free,)

Polymer Detection System with Super Sensitivity and Specificity

|                | Catalog No. | D69-110 | 110 ml (bulk, w/o chromogen) |
|----------------|-------------|---------|------------------------------|
| Storage: 2-8°C |             | D69-18  | 18 ml (w/o chromogen)        |
|                |             | D69-6   | 6 ml (w/o chromogen)         |

## **Intended Use:**

Polink-2 Plus AP Mouse Detection Kit is the 3rd generation of polymer detection system. It uses mouse antibody enhancer 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 further dilute primary antibody due to super sensitivity of Polink-2 Plus detection system. It is a biotin-free system, therefore it 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.

Polink-2 Plus AP 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:  Mouse Antibody Enhancer (Ready-to-use) | Reagent 2:<br>Polymer AP for Mouse<br>(Ready-to-use) |
|-------------|---------------------------------|--|--|
| D69-110     | Polink-2 Plus AP Mouse bulk kit | 110ml  | 110ml  |
| D69-18      | Polink-2 Plus AP Mouse 18ml kit | 18ml   | 18ml   |
| D69-6       | Polink-2 Plus AP Mouse 6ml kit  | 6ml  | 6ml  |

#### **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.
- 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  |             |
|----------------------------|---|-------------|
|                            |   | Time (Min.) |
| 1. HIER PRETREATMENT:      | MENT: 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 2 min., 3 times.   |             |
| 2. PRIMARY ANTIBODY        | a. Apply 2 drops (100 µL) or enough volume of PRIMARY ANTIBODY to cover the tissue            | 30-60       |
| Supplied by user           | section completely. Incubate in moist chamber for 30-60 min.                                  |             |
|                            | b. Rinse with PBS for 2 min., 3 times.  |             |
| 3. Mouse Antibody Enhancer | a. Apply 2 drops (100 μL) or enough volume of MOUSE ANTIBODY ENHANCER to cover                |             |
| (Ready-to-use). Reagent 1  | each section. Incubate in moist chamber for 10 min.   |             |
|                            | b. Rinse with PBS for 2 min., 3 times.  |             |

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| 4. POLYMER-AP for Mouse         | a. Apply 2 drops (100 µL) or enough volume of POLYMER-AP for Mouse to cover each         | 10              |  |
|---------------------------------|--|-----------------|--|
| (Ready-to-use)                  | section. Incubate in moist chamber for 10 min.   |                 |  |
| Reagent 2                       | b. Rinse with PBS for 2 min, 3 times.  |                 |  |
|                                 | c. Rinse with tap water.   |                 |  |
| 5. Chromogen: Supplied by user. | Recommended products:  |                 |  |
| Supplied by user.               | a. Fast-Red kit (Cat. No. C03-60) good for 600 slides                                    |                 |  |
|                                 | b. AP-Red+ kit (Cat. No. C04-8) 40x good for 2000 slides                                 |                 |  |
|                                 | c. BCIP/NBT (RTU) kit (Cat. No. C05-100, C05-18)   |                 |  |
| 6. Hematoxylin:                 | a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and | 20-30           |  |
| Supplied by user.               | wait about 20 seconds.   | seconds         |  |
|                                 | b. Rinse well with tap water for 1-2 min.  |                 |  |
|                                 | c. Put slides in PBS until the color turn blue (about ½ - 1 min.)                        |                 |  |
|                                 | d. Rinse in distill water, then rinse well with tap water                                |                 |  |
| 7. Mounting medium:             | Follow the manufacture data sheet procedure for mounting.                                | Refer to insert |  |
| Supplied by user                | Recommended product:   |                 |  |
|                                 | 1. GB-Mount: Cat. No. E01-18 (18ml), for alcohol soluble substrates (AEC, AP-Red and     |                 |  |
|                                 | AP-blue)   |                 |  |
|                                 | 2. Simpo-Mount: Cat.No. E03-18 (18ml), E03-100 (100ml), universal permanent mounting     |                 |  |
|                                 | medium. Can be used with or without cover slip   |                 |  |

## **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 P   | lus Mouse-NR AP bulk   |                 |               |
| Polink-2 Plus AP Broad Bulk kit          | D68-110        | 110ml      | kit (No cros | ss react to RAT)       | D65-110         | 110ml         |
|  |                |            | Polink-2 P   | lus AP Mouse-NR        |                 |               |
| Polink-2 Plus AP Broad 18ml kit / 6ml    |                |            | 18ml/6ml k   | kit (No cross react to |                 |               |
| kit                                      | D68-18 / D68-6 | 18ml / 6ml | RAT)         |                        | D65-18 / D65-6  | 18ml / 6ml    |
| Polink-2 Plus AP Rabbit bulk kit         | D70-110        | 110ml      | Fast Red k   | Kit                    | C03-60          | 12 Tab + 60ml |
| Polink-2 Plus AP Rabbit 18ml kit / 6ml   |                |            | AP-Red+ k    | Kit (40x concentrate)  | C04-8           | 8ml           |
| kit                                      | D70-18 / D70-6 | 18ml / 6ml |              |                        |                 |               |
| Polink-2 Plus AP Goat bulk               | D66-110        | 110ml      | BCIP/NBT     | Kit                    | C05-100/C05-18  | 100ml / 18ml  |
| Polink-2 Plus AP Goat 18ml kit / 6ml kit | D66-18 / D66-6 | 18ml / 6ml | GB-Mount     | (Aqueous)              | E01-18          | 18ml          |
| Polink-2 Plus AP Rat-NM (no cross        |                |            | Simpo-Mo     | unt (Aqueous)          | E03-100 /E03-18 | 100ml / 18ml  |
| react to mouse) bulk                     | D67-110        | 110ml      |              |                        |                 |               |
| Polink-2 Plus AP Rat-NM (no cross        |                |            |              |                        |                 |               |
| react to mouse) 18ml kit / 6ml kit       | D67-18 / D67-6 | 18ml / 6ml |              |                        |                 |               |

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

**Remarks:** For research use only.

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