

## Polink-1 AP Detection System for Mouse Antibody

(Polymer-AP detection system, biotin-free, Anti-mouse primary antibody)

### Ready-to-use One Step Polymer Detection System

Storage: 4-8°C

|             |                                  |                              |
|-------------|----------------------------------|------------------------------|
| Catalog No. | <input type="checkbox"/> D18-110 | 110 ml (bulk, w/o chromogen) |
|             | <input type="checkbox"/> D18-18  | 18 ml (w/o chromogen)        |
|             | <input type="checkbox"/> D18-6   | 6 ml (w/o chromogen)         |

#### Intended Use:

Polink-1 AP Mouse Detection Kit is designed to use with user supplied mouse antibody to detect target antigen on human tissue or cell samples. Specimen can be frozen or paraffin-embedded tissues, and freshly prepared monolayer cell smears.

Polink-1 AP Mouse Detection Kit is the ONE step polymer detection system that uses polymeric alkaline phosphatase (AP) -linked goat anti mouse IgG to directly detect primary antibody that bound to the tissue. This technology provides excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin<sup>1</sup>. It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2<sup>nd</sup> antibody, and then streptavidin-AP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving.

#### Kit components:

| Catalog No. | Product Name            | Reagent:<br>Polymer AP-linked anti-mouse IgG<br>(Ready-to-use) |
|-------------|-------------------------|--|
| D18-110     | Polink-1 Mouse Bulk kit | 110ml  |
| D18-18      | Polink-1 Mouse 18ml kit | 18ml   |
| D18-6       | Polink-1 Mouse 6ml kit  | 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 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. Proceed IHC staining: DO NOT let specimen or tissue dry from this point on.

| Reagent  | Staining Procedure  | Incubation Time              |
|--|---|------------------------------|
| 1. HIER Pretreatment:<br>Refer to antibody data sheet.         | a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor.<br>b. Wash with PBS 3 times for 2 minutes each time.   | Refer to vendor's data sheet |
| 2. Pre-Block (Optional)<br>Not provided                        | a. Add 2 (100 $\mu$ L) or more drops of 10% Normal Goat Serum to cover the tissue section and Incubate 10 min.<br>b. Drain or blot off solution. DO NOT RINSE.  | 10 min.                      |
| 3. Primary antibody:<br><br>Supplied by user                   | <b>Notes:</b> Investigator needs to optimize dilution and incubation times<br>a. Apply 2 (100 $\mu$ L) or more drops of primary antibody to cover the tissue completely. Incubate in moist chamber for 30-60 min.<br>b. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time. | 30-60 min.                   |
| 4. <b>Reagent:</b><br>AP Polymer-anti-Mouse IgG (Ready-to-use) | a. Apply 2 (100 $\mu$ L) or more drops of AP Polymer-anti-Mouse IgG to cover tissue section and Incubate in moist chamber for 10-15 min.<br>c. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each  | 20-30 min.                   |

|   |  |                      |
|---|--|----------------------|
|   | time.<br>d. Rinse with tap water.  |                      |
| 5. <b>Chromogen:</b><br>Supplied by user.   | Recommended products:<br>a. Fast-Red kit (Cat. No. C03-60) good for 600 slides<br>b. AP-Red+ kit (Cat. No. C04-8) 40x good for 2000 slides<br>c. BCIP/NBT RTU kit (Cat. No. C05-100, C05-18)   |                      |
| 6. Hematoxylin:<br><br>Supplied by user.    | a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about 20 <b>seconds</b> .<br>b. Rinse well with tap water for 1-2 min.<br>c. Put slides in PBS until the color turn blue (about ½ - 1 min.)<br>d. Rinse in distill water, then rinse well with tap water   | 20-30 <b>seconds</b> |
| 7. Mounting medium:<br><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. O-Mount: Cat. No. E02-18 (18ml), for DAB and BCIP/NBT<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 affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting the result.
2. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.

#### Related Products:

| Product                           | Catalog No.    | Size       |  | Product                              | Catalog No.     | Size          |
|-----------------------------------|----------------|------------|--|--------------------------------------|-----------------|---------------|
| Polink-1 AP Broad Bulk kit        | D17-110        | 110ml      |  | **Polink-1 AP Mouse-NR Bulk kit      | D57-110         | 110ml         |
| Polink-1 AP Broad 18ml, 6ml Kit   | D17-18 / D17-6 | 18ml / 6ml |  | **Polink-1 AP Mouse-NR 18ml, 6ml Kit | D57-18 / D57-6  | 18ml / 6ml    |
| Polink-1 AP Rabbit Bulk kit       | D19-110        | 110ml      |  | Fast Red Kit                         | C03-60          | 12 Tab + 60ml |
| Polink-1 AP Rabbit 18ml, 6ml Kit  | D19-18 / D19-6 | 18ml / 6ml |  | AP-Red+ Kit (40x concentrate)        | C04-8           | 8ml           |
| Polink-1 AP Goat Bulk kit         | D61-110        | 110ml      |  | BCIP/NBT Kit                         | C05-100/C05-18  | 100ml / 18ml  |
| Polink-1 AP Goat 18ml, 6ml Kit    | D61-18 / D61-6 | 18ml / 6ml |  | GB-Mount (Aqueous)                   | E01-18          | 18ml          |
| *Polink-1 AP Rat-NM Bulk kit      | D62-110        | 110ml      |  | Simpo-Mount (Aqueous)                | E03-100 /E03-18 | 100ml / 18ml  |
| *Polink-1 AP Rat-NM 18ml, 6ml Kit | D62-18 / D62-6 | 18ml / 6ml |  |                                      |                 |               |

\*Polink-1 AP Rat-NM kit does not cross react with mouse primary antibody

\*\*Polink -1 AP Mouse-NR kit does not cross react with Rat primary antibody

#### Precautions:

Please wear gloves and take other necessary precautions.

#### Remarks:

For research use only.

#### References:

1. Bisgaard K, Pluzed KP. *Use of polymer conjugates in immunohistochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates.* Abstract XXI Intl Cong Intl Acad Pathol and 12<sup>th</sup> World Cong Acad Environ Pathol. Budapest, Hungary, October 20-25, 1996.
2. Shi ZR, Itzkowitz SH, Kim YS. *A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcimoma tissues.* J Histochem Cytochem 36:317-322,