

## Polink-1 AP Mouse-NR (No cross react to RAT) Detection System for IHC

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

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

**Clean background when detect MOUSE antibody on RAT tissue**

Storage: 4-8°C

Catalog No.     D57-110    110 ml (bulk, w/o chromogen)  
                        D57-15     15 ml (w/o chromogen)  
                        D57-6      6 ml (w/o chromogen)

### Intended Use:

Detecting MOUSE primary antibody on RAT tissue is a very difficult task in research field due to background staining issues. Polink-1 AP Mouse-NR Detection Kit is specially designed to solve the problem. The secondary antibody is adsorbed to rat, rabbit and human serum proteins. This technology provides excellent specificity to detect mouse primary antibody (user supplied) on rat tissue. Besides rat tissue Polink-1 AP Mouse-NR Detection Kit also can be used on human tissue as well.

Polink-1 AP Mouse-NR Detection Kit is the ONE step polymer detection system that uses polymeric alkaline phosphatase (AP) -linked anti mouse IgG to directly detect mouse 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: Polymer AP-linked anti-mouse IgG (Ready-to-use)
D57-110	Polink-1 AP Mouse-NR Bulk Kit	110ml
D57-15	Polink-1 AP Mouse-NR 15ml kit	15ml
D57-6	Polink-1 AP Mouse-NR 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: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. b. Wash with PBS 3 times for 2 minutes each time.	Refer to vendor's data sheet
2. Blocking (Optional) Not provided	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum to cover the tissue section and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE.	10 min.
3. Primary antibody: Supplied by user	<b>Notes:</b> Investigator needs to optimize dilution and incubation times a. Apply 2 (100 µL) or more drops of primary antibody to cover the tissue completely. Incubate in moist chamber for 30-60 min. b. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.	30-60 min.
4. <b>Reagent:</b> AP	a. Apply 2 (100 µL) or more drops of AP Polymer-anti-mouse IgG to cover	20-30 min.

Polymer-anti-Mouse IgG (Ready-to-use)	tissue section and Incubate in moist chamber for 20-30 min. c. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.	
5. Chromogen: Supplied by user.	Recommended products: 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, C05-15)	
6. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about 20 <b>seconds</b> . 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	20-30 <b>seconds</b>
7. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. GB-Mount: Cat. No. E01-15 (15ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. O-Mount: Cat. No. E02-15 (15ml), for DAB and BCIP/NBT 3. Simpo-Mount: Cat.No. E03-15 (15ml), 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 Rat-NM Bulk kit	D62-110	110ml
Polink-1 AP Broad 15ml, 6ml Kit	D17-15 / D17-6	15ml / 6ml	**Polink-1 AP Rat-NM 15ml, 6ml Kit	D62-15 / D62-6	15ml / 6ml
Polink-1 AP Rabbit Bulk kit	D19-110	110ml	Fast Red Kit	C03-60	12 Tab + 60ml
Polink-1 AP Rabbit 15ml, 6ml Kit	D19-15 / D19-6	15ml / 6ml	AP-Red+ Kit (40x concentrate)	C04-8	8ml
Polink-1 AP Goat Bulk kit	D61-110	110ml	BCIP/NBT Kit	C05-100/C05-15	100ml / 15ml
Polink-1 AP Goat 15ml, 6ml Kit	D61-15 / D61-6	15ml / 6ml	GB-Mount (Aqueous)	E01-15	15ml
Polink-1 AP Mouse Bulk kit	D18-110	110ml	Simpo-Mount (Aqueous)	E03-100 /E03-15	100ml / 15ml
Polink-1 AP Mouse 15ml, 6ml Kit	D18-15 / D18-6	15ml / 6ml			

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

#### Precautions:

Please wear gloves and take other necessary precautions.

#### Remarks:

For research use or investigation only. Not for diagnostic or therapeutic use.

#### 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 carcinoma tissues.* J Histochem Cytochem 36:317-322,