Polink-1 HRP Rat-NM AEC Detection System for Mouse Primary Antibody

(Polymer-HRP detection system, biotin-free, Anti-rat primary antibody)

Ready-to-use One Step Polymer Detection System
Supper clean when using rat antibody on mouse tissue
Super Sensitive for AEC Staining

Storage: 4-8ºC

Catalog No.
- ☐ D36-110 110 ml (bulk, w/o chromogen)
- ☐ D36-18 18 ml (with AEC, good for 180 slides)
- ☐ D36-6 6 ml (with AEC, good for 50 slides)
- ☐ D36-Sample 3 ml (with AEC, good for 25 slides)

Intended Use:

Detecting RAT primary antibody on MOUSE tissue is a very difficult task in research field due to background issues. Polink-1 HRP Rat-NM (no-mouse) AEC Detection kit is specially designed to solve the problem. This technology provides excellent specificity to detect rat primary antibody (user supplied) on mouse tissue. Specimen can be frozen or paraffin – embedded tissues, and freshly prepared monolayer cell smears. This detection system is super sensitive when use with AEC chromogen.

Polink-1 HRP Rat-NM AEC Detection kit is the ONE step polymer detection system that uses polymeric HRP-linked anti rat secondary antibody to directly detect rat primary antibody bound to the mouse tissue. The secondary antibody was adsorbed to mouse, rabbit and human serum proteins. Besides mouse tissue Polink-1 HRP Rat-NM AEC Detection kit also can be used on human tissue and rabbit tissue as well. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin[1]. It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2nd antibody, and then streptavidin-HRP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving.

If user needs most sensitive polymer detection system for rat primary antibody on mouse tissue, one may choose two-step polymer detection system, Polink-2 Plus HRP Rat-NM AEC kit (Cat No. D48-110, D48-18, D48-6).

Kit components:

Catalog No.	Product Name	Reagent 1: Polymer HRP-linked anti-rat IgG for AEC (Ready-to-use)	Reagent 2: 2A: substrate buffer (20x) 2B: Chromogen (20x) 2C: H_2O_2 (20x)
D36-110	Polink-1 HRP Rat-NM Bulk for AEC kit	110ml	Not provided
D36-18	Polink-1 HRP Rat-NM AEC 18ml kit	18ml	3ml of Reagent 2A 6ml of Reagent 2B 3ml of Reagent 2C
D36-6	Polink-1 HRP Rat-NM AEC 6ml kit	6ml	2ml of Reagent 2A 4ml of Reagent 2B 2ml of Reagent 2C

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 (Min.)
1. Peroxidase Blocking Reagent Supplied by user	a. Incubate slides in peroxidese blocking reagent (Ready-to-use 3% H_2O_2 solution) for 10 min. b. Rinse the slide using distilled water.	10
2. 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
3. Pre-Block (Optional)	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum to cover the tissue section	10

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Not provided	and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE.	
4. Primary antibody: Supplied by user	***Notes:*** 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
5. **Reagent 1:** HRP Polymer-anti-rat (x mouse) RTU	a. Apply 2 (100 µL) or more drops of HRP Polymer-anti-Rat IgG to cover tissue section and Incubate in moist chamber for 10-15 min. c. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.	10-15
6. **Reagents 2A, 2B and 2C:** AEC Chromogen (20x)	a. Add 1 drop of Reagent 2A, 1 drop or 2 drop (for high contrast) of Reagent 2B and 1 drop of Reagent 2C to 1 mL distilled or deionized water. Mix well. Protect from light and use within one hour. b. Apply 2 drops (100 µL) or enough volume of pre-mixed AEC Chromogen to completely cover tissue. Incubate for 5 min. to 10 min.. c. Rinse thoroughly with distill water	3-10
8. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about 20 **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	20-30 **seconds**
9. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. GB-Mount: Cat. No. E01-18 (18ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. O-Mount: Cat. No. E02-18 (18ml), for DAB and BCIP/NBT 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 HRP Mouse Bulk kit for AEC	D15-110	110ml		*Polink-1 HRP Mouse-NR 18ml, 6ml AEC Kit	D56-18 / D56-6	18ml / 6ml
Polink-1 HRP Mouse 18ml, 6ml AEC Kit	D15-18 / D15-6	18ml / 6ml		Polink-1 HRP Broad Bulk kit for AEC (for mouse & rabbit antibody)	D14-110 / D14-60	110ml/ 60ml
Polink-1 HRP Rabbit Bulk kit for AEC	D16-110	110ml		Polink-1 HRP Broad 18ml, 6ml AEC Kit (for mouse & rabbit antibody)	D14-18 / D14-6	18ml / 6ml
Polink-1 HRP Rabbit 18ml, 6ml AEC Kit	D16-18 / D16-6	18ml / 6ml		AEC Kit	C01-12	12ml
Polink-1 HRP Goat Bulk kit for AEC	D34-110	110ml		GB-Mount (Aqueous)	E01-18	18ml
Polink-1 HRP Goat 18ml, 6ml AEC Kit	D34-18 / D34-6	18ml / 6ml		Simpo-Mount (Aqueous)	E03-100 /E03-18	100ml/18ml
*Polink-1 HRP Mouse-NR Bulk kit for AEC	D56-110	110ml				

*Polink-1 HRP Mouse-NR kit does not cross react with mouse primary antibody

Precautious:
Please wear gloves and take other necessary precautions.

Remarks:
For research use only.

References:
1. Bisgaard K, Pluzed KP. *Use of polymer conjugates in immunohitochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates.* Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungry, 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 Hitochem Cytochem 36:317-322,

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