GBI Labs
GOLDEN BRIDGE INTERNATIONAL

Polink-1 HRP Mouse-NR (No cross react with Rat)
DAB Detection System for Mouse Primary Antibody

(Polymer-HRP detection system, biotin-free, detect mouse primary antibody)
Ready-to-use One Step Polymer Detection System
Super clean when using mouse antibody on rat tissue

Storage: 4-8ºC

Catalog No.
☐ D55-110 110 ml (bulk, w/o chromogen)
☐ D55-18 18 ml (with DAB, good for 180 slides)
☐ D55-6 6 ml (with DAB, good for 50 slides)

Intended Use:

Detecting MOUSE primary antibody on RAT tissue is a very difficult task in research field due to background staining issues. Polink-1 HRP Mouse-NR (No-Rat) DAB Detection kit is specially designed to solve the problem. This technology provides excellent specificity to detect mouse primary antibody (user supplied) on rat tissue. Specimen can be frozen tissues, paraffin–embedded tissues, or freshly prepared monolayer cell smears.

Polink-1 HRP Mouse-NR DAB Detection kit is a 1-step polymer detection system that uses polymeric HRP-linked anti-mouse secondary antibody to directly detect mouse primary antibody bound to the rat tissue. The secondary antibody is adsorbed to rat, rabbit and human serum proteins. Besides rat tissue Polink-1 HRP Mouse-NR DAB 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 endogenous biotin[1]. It is a 1-step detection system is a much faster assay compared to traditional two step methods (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 users need a more sensitive polymer detection system for mouse primary antibody on rat tissue, they may choose a two-step polymer detection system, Polink-2 Plus HRP Mouse-NR DAB kit (Cat No. D58-110, D58-18, D58-6). For AEC staining please choose Polink-1 HRP Mouse-NR for AEC (D56-110, D56-18, and D56-6).

Kit components:

Catalog No.	Product Name	Reagent 1: Polymer HRP-linked anti-mouse IgG (No cross react with rat) (Ready-to-use)	Reagent 2: 2A: DAB Substrate 2B: Chromogen concentrate
D55-110	Polink-1 HRP Mouse-NR Bulk for DAB kit	110ml	Not provided
D55-18	Polink-1 HRP Mouse-NR with DAB 18ml kit	18ml	30 ml of 2A and 2 ml of 2B
D55-6	Polink-1 HRP Mouse-NR with DAB 6ml kit	6ml	12 ml of 2A and 1.5 ml of 2B

Recommended Protocol:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, users need to supply appropriately fixed tissue and well prepared slides.
2. Tissue needs 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 peroxidase blocking reagent (Ready-to-use 3% H_2O_2 solution) for 10 minutes. 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 for 2 minutes each time for 3 times.	Refer to vendor's data sheet
3. Pre-Block (Optional) Not provided	a. Add 2 (100 µL) or more drops of Pre-Block solution to cover the tissue section and Incubate b. Drain or blot off solution. DO NOT RINSE.	10

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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 for 2 minutes each time for 3 times.	30-60
5. **Reagent 1:** HRP Polymer-anti-mouse (Ready-to-use)	a. Apply 2 (100 µL) or more drops of HRP Polymer-anti-Mouse 2nd antibody to cover tissue section and Incubate in moist chamber for 10-15 min. c. Rinse with PBS containing 0.05% Tween-20 for 2 minutes each time for 3 times.	10-15
6. **Reagents 2A, 2B:** 2A: DAB Substrate 2B: DAB Chromogen	a. Adding 1 drop or 2 drops (for higher sensitivity and contrast) of DAB chromogen concentrate (Reagent 2B) in 1ml of DAB substrate buffer (Reagent 2A). Mix well. b. Apply 2 drops (100 µL) or enough volume of pre-mixed DAB Chromogen to completely cover tissue. Incubate for 5 min. Use the prepared DAB solution within 5 hours. c. When appropriate color is developed, rinse under tap water gently for about 1-2 minutes.	3-10
7. 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**
8. 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. Users need 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 Broad Bulk kit for DAB	D11-110	110ml		Polink-1 HRP Mouse 18ml, 6ml DAB Kit	D12-18 / D12-6	18ml / 6ml
Polink-1 HRP Broad 18ml, 6ml DAB Kit	D11-18 / D11-6	18ml / 6ml		Polink-1 HRP Rat-NM (no cross react to mouse) Bulk kit for DAB	D35-110	110ml
Polink-1 HRP Rabbit Bulk kit for DAB	D13-110	110ml		Polink-1 HRP Rat-NM (no cross react to mouse)18ml, 6ml DAB Kit	D35-18 / D35-6	18ml / 6ml
Polink-1 HRP Rabbit 18ml, 6ml DAB Kit	D13-18 / D13-6	18ml / 6ml		DAB Kit (2-components)	C09-12	12ml +240ml
Polink-1 HRP Goat Bulk kit for DAB	D33-110	110ml		O-Mount (Organic)	E02-18	18ml
Polink-1 HRP Goat 18ml, 6ml DAB Kit	D33-18 / D33-6	18ml / 6ml		Simpo-Mount (Aqueous)	E03-100/ E03-18	100ml / 18ml
Polink-1 HRP Mouse Bulk kit for DAB	D12-110	110ml				

*Polink -1 HRP Rat-NM kit is designed for rat primary antibody on mouse tissue.

Precautious:
AEC may be carcinogenic. 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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