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

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

Ready-to-use One Step Polymer Detection System

Super clean when using rat antibody on mouse tissue

Storage: 4-8ºC

Catalog No.

- ☐ D35-110 — 110 ml (bulk, w/o chromogen)
- ☐ D35-18 — 18 ml (with DAB, good for 180 slides)
- ☐ D35-6 — 6 ml (with DAB, good for 50 slides)

Intended Use:

Detecting RAT primary antibody on MOUSE tissue is a challenging task in research due to background staining issues. GBI Labs' Polink-1 HRP Rat-NM (No-Mouse) DAB Detection kit is specially designed to overcome these inter-species background staining issues. This technology provides excellent specificity to detect rat primary antibody (user supplied) on mouse tissue. Specimens can be frozen, paraffin embedded tissues, or freshly prepared cell smears made up to a monolayer.

Polink-1 HRP Rat-NM DAB Detection kit is a 1-step polymer detection system that uses polymeric HRP-linked anti-rat secondary antibody to directly detect rat primary antibody bound on mouse tissue. The secondary antibody is adsorbed to mouse, rabbit and human serum proteins. The Polink-1 HRP Rat-NM DAB Detection kit is suitable for human and rabbit tissue as well. It is a biotin-free system, overcoming the non-specific staining caused by endogenous biotin[1]. As a 1-step detection system, it can be completed much faster compared to traditional two step methods (Biotinylated 2nd antibody, and then streptavidin-HRP). These advantages provide laboratories the benefit of cost effective, faster and more accurate results with less trouble shooting.

If users need a more sensitive polymer detection system for rat primary antibody on mouse tissue, they may choose a two-step polymer detection system, Polink-2 Plus HRP Rat-NM DAB kit (Cat No. D46-110, D46-18, D46-6). For AEC staining please choose Polink-1 HRP Rat-NM for AEC (D36-110, D36-18, and D36-6).

Kit components:

Catalog No.	Product Name	Reagent 1: Polymer HRP-linked anti-Rat IgG (No cross react with mouse) (Ready-to-use)	Reagent 2: 2A: DAB Substrate 2B: Chromogen concentrate
D35-110	Polink-1 HRP RAT-NM Bulk for DAB kit	110ml	Not provided
D35-18	Polink-1 HRP RAT-NM with DAB 18ml kit	18ml	30 ml of 2A and 2 ml of 2B
D35-6	Polink-1 HRP RAT-NM 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.
8. Serum blocking before primary antibody incubation for GBI's Polink-1, Polink-2, and Polink-2Plus is not required because all our antibody conjugates are absorbed to human serum.

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-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	Refer to vendor's data sheet

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3. Pre-Block (Optional) Not provided	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (E07) to cover the tissue section and Incubate b. Drain or blot off solution. DO NOT RINSE. c. See note 8 in Recommended Protocol.	10
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. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	30-60
5. **Reagent 1:** Polymer-HRP anti-rat (Ready-to-use)	a. Apply 2 (100 µL) or more drops of Polymer- HRP anti-Rat 2nd antibody to cover tissue section and Incubate in moist chamber for 15-30 min. **We recommend incubating the polymer up to 30mins for best sensitivity.** b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	15-30
6. **Reagents 2A, 2B:** 2A: DAB Substrate 2B: DAB Chromogen	a. Adding 1 drop or 2 drops (for higher 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
8. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100 µl) 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. 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 Mouse-NR Bulk kit for DAB	D55-110	110ml
Polink-1 HRP Rabbit Bulk kit for DAB	D13-110	110ml		*Polink-1 HRP Mouse-NR 18ml, 6ml DAB Kit	D55-18 / D55-6	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 Mouse-NR kit does not cross react with Rat primary antibody

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.

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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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