NeoStain Poly 1-Step Kit, HRP Detection System for Rat Antibodies (for DAB)

NB-23-00032

NeoStain Poly 1-Step Kit, Horseradish peroxidase Rat-NM (No cross react with Mouse) Detection System Kit forRat Primary Antibodies (for DAB)

(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

#Cat: NB-23-00032-1 **Size: 110mL, no chromogen**
#Cat: NB-23-00032-2 **Size: 18ml, with DAB** (good for 150 slides)
#Cat: NB-23-00032-3 **Size: 6ml, with DAB** (good for 50 slides)

Intended Use:

Detecting RAT primary antibody on MOUSE tissue is a very difficult task in research field due to background staining issues. NeoStain Poly 1-Step HRP Rat-NM (No-Mouse) DAB 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 tissues, paraffin-embedded tissues,or freshly prepared monolayer cell smears.

NeoStain Poly 1-Step 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 to the mouse tissue. The secondary antibody is adsorbed to mouse, rabbit and human serum proteins. Besides mouse tissue Poly 1-Step HRP Rat-NM DAB Detection kit also can be used on human tissueand 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 streptavidinHRP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better costsaving.

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, NeoStain Poly 2-Step HRP Rat-NM DAB kit Cat# NB-23-00052-1 (110ml) /-2 (18ml) /-3 (6ml). For AEC staining please choose NeoStain Poly 1-Step HRP Rat-NM for AEC Cat# NB-23-00038- (110ml /-2 (18ml) /-3 (6ml).

Kit Components:

Catalog No.	**Product Name**	**Reagent 1:** Polymer HRP-linked anti-rat IgG(Ready-to-use)	**Reagent 2:** 2A: DAB Substrate 2B: Chromogen concentrate
NB-23-00032-1	NeoStain Poly 1-Step no chromogen	110ml	Not provided
NB-23-00032-2	NeoStain Poly 1-Step with DAB	18ml	30 ml of 2A and 2 ml of 2B
NB-23-00032-3	NeoStain Poly 1-Step with DAB	6ml	12 ml of 2A and 1.5 ml of 2B

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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:

Reagent	**Staining Procedure**	**Incubation Time (Min.)**
1. Peroxidase Blocking Reagent Supplied by user	a. Incubate slides in peroxidese blocking reagent (Ready-to-use 3%H2O2 solution) for 10 min. b. Rinse the slide using distilled water.	10
2. HIER Pretreatment: Refer to antibody data sheet.	eat Induced Epitope Retrieval (HIER) may be required for rimary antibody suggested by vendor. b. Wash with PBS 3 times for 2 minutes each time.	Refer to vendor's datasheet
3. Pre-Block (Optional)Not provided	a. Add 2 (100 µL) or more drops of Pre-Block solution to cover thetissue section and Incubate. b. Drain or blot off solution. DO NOT RINSE Drain.	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. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minuteseach time.	30-60
5. **Reagent 1:** HRP Polymer-anti-Rat IgG	a. Apply 2 (100 µL) or more drops of Polymer-HRP anti-Rat 2nd antibody to cover tissue section and Incubate in moist chamberfor 10-15 min. b. c. Rinse with PBS containing 0.05% Tween-20 for 2 minutes each time for 3 times	15
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 substratebuffer (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. usethe prepared DAB solution within 5 hours c. When appropriate color is developed, rinse under tap watergently for about 1-2 minutes.	3-10

8. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about20 **seconds**. b. Rinse well with tap water for 1-2 min. c. Put slides in PBS until the color turn blue (about 15-30 seconds.) d. Rinse in distill water, then rinse well with tap water	20-30 **seconds**
9. Mounting medium:Supplied by user	Recommended product: 1. NeoBio Mount AQ: Cat.# NB-00155-3 (18ml), for alcohol solublesubstrates (AEC, AP-Red and AP-blue) 2. NeoBio Mount Perm: Cat.# NB-23-00156, for DAB & BCIP/NBT NeoBio Mount Universal: Cat.# NB-23-00157-2 (18ml), orNB-23-00157-1 (100ml), universal permanent mounting medium. Can be used with or without cover slip	Follow the manufacturedata sheet procedure for mounting . 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.

Precautious:
DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

Remarks:
For research use only.

Storage:
Store at 4°C.

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,

Related products

Product	Catalog No.	Size
NeoStain Poly 1-Step HRP Mouse/Rabbit Bulk kit for DAB	NB-23-00028-1	1L
NeoStain Poly 1-Step HRP Mouse/Rabbit 18ml, 6ml DAB Kit	NB-23-00028-4 / -5	18ml / 6ml
NeoStain Poly 1-Step Mouse Bulk kit for DAB	NB-23-00029-1	110ml
NeoStain Poly 1-Step Mouse 18ml, 6ml DAB Kit	NB-23-00029-4 / -5	18ml / 6ml
NeoStain Poly 1-Step Goatt Bulk kit for DAB	NB-23-00031-1	110ml
NeoStain Poly 1-Step Goat 18ml, 6ml DAB Kit	NB-23-00031-2 / -3	18ml / 6ml
NeoStain Poly 1-Step HRP Rat-NM Bulk kit for DAB (no x Mouse)	NB-23-00032-1	110ml
NeoStain Poly 1-Step HRP Rat-NM 18ml, 6ml DAB Kit (no x Mouse)	NB-23-00032-2 / -3	18ml / 6ml
NeoStain Poly 1-Step HRP Mouse-NR Bulk kit for DAB (no x Rat)	NB-23-00033-1	110ml
NeoStain Poly 1-Step HRP Mouse-NR 18ml, 6ml DAB Kit (no x Rat)	NB-23-00033-2 / -3	18ml / 6ml
DAB+ 2 components	NB-23-00148-1	12ml +240ml
NeoBio Mount Perm (Organic)	NB-23-00156	18ml
NeoBio Mount Universal (Aqueous)	NB-23-00157-1 / -2	100ml / 18ml