



NeoStain Poly 2-Step Kit,
HRP for DAB, Mouse &
Rabbit, with DAB

NB-23-00204-4

NB-23-00204-5

NeoStain Poly 2-Step Kit, HRP for DAB, Mouse & Rabbit, with DAB

#Cat: NB-23-00204-4

Size: 110mL

#Cat: NB-23-00204-5

Size: 60mL

Intended Use:

NeoStain Poly 2-Step Kit is a HRP polymer based detection system that can be used to detect mouse or rabbit primary antibodies for immunohistochemical screens of human tissues. NeoStain Poly 2-Step Kit are a second generation polymer detection system that uses polymer helper and polymeric HRP-linked antibody conjugates to get consistent immunostaining outcomes on archival tissues and to increase sensitivity on difficult-to-work antibodies, whether the antigen is cytoplasmic, membranous, or nuclear. 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. This kit has been tested on paraffin embedded tissue only; however it may be used on frozen or freshly prepared monolayer cell smears.

Kit Components:

| Catalog No. | Reagent 1 Polymer Helper (RTU) | Reagent 2: Polymer HRP- linked anti- mouse IgG (RTU) | Reagent 3A DAB Substrate (RTU) | Reagent 3B: DAB Chromogen (20x) |
|---------------|--------------------------------------|---|--------------------------------------|---------------------------------------|
| NB-23-00204-4 | 110mL | 110mL | 110mL | 6mL |
| NB-23-00204-5 | 60mL | 60mL | Not provided | Not provided |

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.
8. Serum blocking before primary antibody incubation for NeoStain Poly 1-Step, NeoStain Poly 2-Step, and NeoStain Poly 2-Step Plus is not required because all our antibody conjugates are absorbed to human serum

Reagent:

| 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 ₂ O ₂ solution) for 10 min. b. Rinse the slide using distilled water. | 10 |
| 2. HIER Pretreatment: Refer to antibody datasheet. | 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 datasheet |
| 3. Pre-Block (Optional) Not provided | a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (NeoBlock, NB-23-00169) to cover the tissue section and incubate 10 min. 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 Helper (RTU) | a. Apply 2 drops (100 µL) or enough volume of Reagent 1 Polymer Helper to cover each section. Incubate in moist chamber for 30 min. b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time. | 30 |
| 6. Reagents 2: Polymer HRP anti-Mouse/Rabbit IgG (RTU) | a. Apply 2 drops (100 µL) or enough volume of Reagent 2 Polymer HRP anti-Mouse/Rabbit IgG to cover each section. Incubate in moist chamber for 15-20 min. b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time. | 15-20 |

| | | |
|---|--|------------------------|
| <p>7. Chromogen Reagent 3A: DAB Substrate(RTU) Reagent 3B: DAB Chromogen(20x)</p> | <p>a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B into 1mL Reagent 3A. Mix well. b. Apply 2 drops (100µL) or enough of DAB working solution to completely cover tissue section. Incubate for about 5 min. Monitor the color development under microscope. c. Rinse well in distill or tap water Note: Protect DAB working solution from light and use within 5 hours.</p> | <p>5</p> |
| <p>8. Hematoxylin: Supplied by user.</p> | <p>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 30-60 seconds) d. Rinse in distill water, then rinse</p> | <p>20-30second</p> |
| <p>9. Mounting medium:Supplied by user</p> | <p>Follow the manufacture data sheet procedure for mounting. Recommended product: 1. NeoBio Mount AQ: Cat.# NB-00155-3 (18ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. NeoBio Mount Perm: Cat.# NB-23-00156 (18ml), for DAB and BCIP/NBT 3. NeoBio Mount Universal: Cat.# NB-23-00157-2 (18ml), or NB-23- 00157-1 (100ml), universal permanent mounting medium. Can be used with or without cover slip</p> | <p>Refer to insert</p> |

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. Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.

Precautious:

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

Remarks:

For research use only.

Storage:

Store at 4°C.

Related products

| Product | Catalog No. | Size |
|---|--------------------|---------------|
| NeoStain Poly 2-Step AP Broad Bulk kit (w/o chromogen) | NB-23-00047-1 | 110ml |
| NeoStain Poly 2-Step AP Broad kit 18ml, 6ml (w/o chromogen) | NB-23-00047-2 / -3 | 18ml / 6ml |
| NeoStain Poly 2-Step HRP Broad Bulk kit for AEC (w/o AEC) | NB-23-00046-1 / -2 | 110ml / 60ml |
| NeoStain Poly 2-Step HRP Broad AEC kit | NB-23-00046-3 / -4 | 18ml / 6ml |
| AEC kit (20x) | NB-23-00140 | 12ml |
| Fast Red Kit | NB-23-00142 | 12 tab + 60ml |
| AP-Red+ Kit (40x) | NB-23-00143 | 8ml |
| BCIP/NBT Kit | NB-23-00144-1 / -2 | 100ml / 18ml |
| DAB+ 2 components | NB-23-00148-1 | 12ml +240ml |
| NeoBio Mount AQ | NB-00155-3 | 18ml |
| NeoBio Mount Perm (Organic) | NB-23-00156 | 18ml |
| NeoBio Mount Universal (Aqueous) | NB-23-00157-1 / -2 | 100ml / 18ml |