



NeoStain Poly 2-Step Plus
Kit HRP

NB-23-00050-2

NB-23-00050-3

NB-23-00050-4

NeoStain Poly 2-Step Plus Kit HRP

#Cat: NB-23-00050-2	Size: 110mL (Bulk, w/o chromogen)
#Cat: NB-23-00050-3	Size: 6mL (with DAB, good for 50 slides)
#Cat: NB-23-00050-4	Size: 18mL (with DAB, good for 180 slides)

Intended Use:

Storage: 4-8°C

NeoStain Poly 2-Step Plus Kit HRP is the 3rd generation of polymer detection system. It uses rabbit antibody specific enhancer to help amplify the polymer enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of NeoStain Poly 2-Step detection system. It is a biotin free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. Suitable for manual or autostain. Staining conditions need to be optimized by user.

NeoStain Poly 2-Step Plus Kit HRP Detection System offers a wide choice for primary antibodies, including broad spectrum (for mouse and rabbit primary antibodies), mouse, rabbit, goat, and rat primary antibodies. Refer to **Related Product** section for details.

Kit components:

Product Name	Product Name	Reagent 1: Rabbit Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Rabbit (Ready-to-use)	Reagent3A, 3B: 3A: DAB Substrate (Ready-to-use) 3B: DAB Chromogen (20xConcentrate)
NB-23-00050-2	NeoStain Poly 2-Step Plus Kit, HRP for DAB, Rabbit, no chromogen	110ml	110ml	110mL
NB-23-00050-3	NeoStain Poly 2-Step Plus Kit, HRP, Rabbit, with DAB	18ml	18mL	110mL
NB-23-00050-4	NeoStain Poly 2-Step Plus Kit, HRP, Rabbit, with DAB	6ml	6mL	12ml of DAB Reagent 3A 1.5ml of DAB Chromgen 3B

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. Staining steps: DO NOT let specimen or tissue dry from this point on.
8. Serum blocking before primary antibody incubation for Neo Biotech's NeoStain-1, NeoStain-2, and NeoStain-2Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent	Staining Procedure	Staining Procedure
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 minutes. b. Rinse the slide using distilled water.	10
2. Hier Pretreatment:	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. Please check the data sheet of primary antibody b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time	
3. Pre-Block (Optional) Supplied by user	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (NB- 23-00169-2) 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.	
4. Primary Antibody Supplied by user	a. Apply 2 drops (100 µL) or enough volume of Primary Antibody to cover the tissue section 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. Rabbit Antibody Enhancer (Ready to-use). Reagent 1	a. Apply 2 drops (100 µL) or enough volume of Rabbit Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30 min. (We recommend incubating the antibody enhancer up to 30mins for best sensitivity) b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time	10-30
6. POLYMER-HRP for Rabbit (Ready-to-use) Reagent 2	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for RABBIT to cover each section. Incubate in moist chamber for 10-30 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.	10-30

<p>7. Chromogen Reagent 3A: DAB Substrate Reagent 3B: DAB Chromogen</p>	<p>a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B into 1ml of reagent 3A. Mix well. Protect from light and use within 5 hours. b. Apply 2 drops (100 µL) or enough to completely cover tissue, of pre-mixed DAB to each section. Incubate for about 5 min. Monitor the color development under microscope. c. Rinse with tap water for 1-2 min.</p>	<p>5</p>
<p>8. Hematoxylin Supplied by user</p>	<p>a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue completely and wait about 15-20 seconds. b. Rinse well under tap water for 1-2 minutes. c. Put slides in PBS until show blue color (about 30-60 seconds). d. Rinse well in distill or tap water</p>	<p>15-20 seconds</p>
<p>9. Mounting medium: Supplied by user</p>	<p>Follow the manufacture data sheet procedure for mounting. Recommended product: 1. NeoBio Mount Perm (Permanent mount for DAB, BCIP/NBT): Cat. No. NB-23-00156 (18ml), for AEC, AP-Red, and AP-blue. 2. NeoBio Mount Perm (Permanent mount for DAB, BCIP/NBT): Cat. No. NB-23-00156 (18ml), for DAB 3. NeoBio Mount Universal: Cat.No. 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 effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret 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.

Related Products:

Product	Catalog No.	Size
NeoStain Poly 2-Step Plus Kit, HRP for DAB, Mouse, no chromogen	NB-23-00049-2	110ml
NeoStain Poly 2-Step Plus Kit, HRP, Mouse, with DAB	NB-23-00049-3 NB-23-00049-4	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, HRP for DAB, Goat, no chromogen	NB-23-00051-2	110ml
NeoStain Poly 2-Step Plus Kit, HRP, Goat, with DAB	NB-23-00051-3 NB-23-00051-4	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, HRP for DAB, Mouse & Rabbit, no chromogen	NB-23-00048-3	110ml
NeoStain Poly 2-Step Plus Kit, HRP, Mouse & Rabbit, with DAB	NB-23-00048-4 NB-23-00048-5	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, HRP for DAB, Rat-NM, no chromogen	NB-23-00052-1	110ml
NeoStain Poly 2-Step Plus Kit, HRP, Rat-NM, with DAB	NB-23-00052-2 NB-23-00052-3	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, HRP for DAB, Mouse-NR, no chromogen	NB-23-00053-1	110ml
NeoStain Poly 2-Step Plus Kit, HRP, Mouse-NR, with DAB	NB-23-00053-2 NB-23-00053-3	18ml 6ml
DAB+ 2 components	NB-23-00148-1/ NB-23-00144-2	12ml +240ml
NeoBio Mount Perm (Permanent mount for DAB, BCIP/NBT)	NB-23-00156	18ml

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

Remarks: For research use only.