



PolyStain 2-Step Kit – HRP for DAB Detection System for Mouse and Rabbit Antibodies

- NB-23-00204-1 (6ml, with DAB)**
- NB-23-00204-2 (1L, no chromogen)**
- NB-23-00204-4 (110ml, with DAB)**
- NB-23-00204-5 (60ml, no chromogen)**
- NB-23-00204-6 (60ml, with DAB)**
- NB-23-00204-7 (18ml, with DAB)**

PolyStain 2-Step Kit – HRP for DAB Detection System for Mouse and Rabbit Antibodies (Broad Spectrum)

***(Polymer-HRP detection system, biotin-free, anti-mouse/rabbit primary antibodies)
Second Generation of Polymer Detection System***

NB-23-00204-1	size : 6ml, with DAB (good for 60 slides)
NB-23-00204-2	size : 1L, no chromogen (good for 10000 slides)
NB-23-00204-4	size : 110ml, with DAB (good for 1800 slides)
NB-23-00204-5	size : 60ml, no chromogen (good for 600 slides)
NB-23-00204-6	size : 60ml, with DAB (good for 600 slides)
NB-23-00204-7	size : 18ml, with DAB (good for 180 slides)

Intended Use:

PolyStain 2-StepHRP DAB Broad-2 Detection 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. PolyStain 2-Stepkits 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-23-00204-1	6mL	6mL	12mL	1.5mL
NB-23-00204-2	1L	1L	Not provided	Not provided
NB-23-00204-4	110mL	110mL	110mL	6mL
NB-23-00204-5	60mL	60mL	Not provided	Not provided
NB-23-00204-6	60mL	60mL	60mL	3mL
NB-23-00204-7	18mL	18mL	15mLx2	2mL

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 PolyStain 1-Step, PolyStain 2-Step, and PolyStain 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	<ol style="list-style-type: none"> a. Incubate slides in peroxidase 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.	<ol style="list-style-type: none"> 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
3. Pre-Block (Optional) Not provided	<ol style="list-style-type: none"> 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	<p>Notes: Investigator needs to optimize dilution and incubation times</p> <ol style="list-style-type: none"> 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)	<ol style="list-style-type: none"> a. Apply 2 drops (100µL) or enough volume of Reagent 1 Polymer Helper to cover each section. Incubate in moist chamber for 30min. 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)	<ol style="list-style-type: none"> a. Apply 2 drops (100µL) 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
7. Chromogen Reagent 3A: DAB Substrate(RTU) Reagent 3B: DAB Chromogen(20x)	<ol style="list-style-type: none"> 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 <p>Note: Protect DAB working solution from light and use within 5 hours.</p>	5
8. Hematoxylin: Supplied by user.	<ol style="list-style-type: none"> 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 well with tap water 	20-30 seconds

<p>9. Mounting medium:</p> <p>Supplied by user</p>	<p>Follow the manufacture data sheet procedure for mounting.</p> <p>Recommended product:</p> <ol style="list-style-type: none"> 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>Refer to insert</p>
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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.

Precautions:

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
PolyStain 2-Step AP Broad Bulk kit (w/o chromogen)	NB-23-00047-1	110ml
PolyStain 2-Step AP Broad kit 18ml, 6ml (w/o chromogen)	NB-23-00047-2 / -3	18ml / 6ml
PolyStain 2-Step HRP Broad Bulk kit for AEC (w/o AEC)	NB-23-00046-1 / -2	110ml / 60ml
PolyStain 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