



PolyStain 2-Step Plus Kit –  
HRP for DAB Detection  
System for Rabbit  
Antibody

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**NB-23-00050-1 (1L, no chromogen)**  
**NB-23-00050-2 (110mL, no chromogen)**  
**NB-23-00050-3 (18ml, with DAB)**  
**NB-23-00050-4 (6ml, with DAB)**

## PolyStain 2-Step Plus Kit - HRP Rabbit DAB Detection System for Immunohistochemistry

(2-step Polymer-HRP detection system, biotin-free)  
Polymer Detection System with Super Sensitivity and Specificity

NB-23-00050-1 size : 1L, no chromogen  
NB-23-00050-2 size : 110mL, no chromogen  
NB-23-00050-3 size : 18ml, with DAB (good for 180 slides)  
NB-23-00050-4 size : 6ml, with DAB (good for 50 slides)

### Intended Use:

PolyStain 2-Step Plus Kit - HRP anti Rabbit Detection Kit 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 PolyStain 2-Step Plus detection system. It is a biotin-free system, therefore, overcomes the non-specific staining caused by endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. It can be used for manual stain or autostainer. Staining conditions need to be optimized by user.

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

### Kit Components:

Catalog No.	Product Name	Reagent 1: Rabbit Antibody Enhancer (RTU)	Reagent 2: Polymer HRP for Rabbit (RTU)	Reagent 3A, 3B: 3A: DAB Substrate (RTU) 3B: DAB Chromogen Concentrate
NB-23-00050-1	PolyStain 2-Step Plus Kit - HRP for DAB Rabbit Bulk	1L	1L	Not included
NB-23-00050-2	PolyStain 2-Step Plus Kit - HRP for DAB Rabbit Bulk	110ml	110ml	Not included
NB-23-00050-3	PolyStain 2-Step Plus Kit - HRP for DAB Rabbit 18ml	18ml	18ml	30ml of DAB Reagent 3A 2ml of DAB Chromgen 3B
NB-23-00050-4	PolyStain 2-Step Plus Kit - HRP for DAB Rabbit 6ml	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.

### Reagent:

Reagent	Staining Procedure	Incubation Time (Min.)
1. PEROXIDASE BLOCKING REAGENT. Supplied by user	<ol style="list-style-type: none"> <li>a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H<sub>2</sub>O<sub>2</sub> solution) for 10 minutes.</li> <li>b. Rinse the slide using distilled water.</li> </ol>	10
2. HIER PRETREATMENT:	<ol style="list-style-type: none"> <li>a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. Please check the data sheet of primary antibody</li> <li>b. Wash with PBS 2 min., 3 times.</li> </ol>	
3. Pre-antibody Blocking: Supplied by user	Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.	
4. PRIMARY ANTIBODY Supplied by user	<ol style="list-style-type: none"> <li>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.</li> <li>b. Rinse with PBS for 2 min., 3 times.</li> </ol>	30-60
5. Rabbit Antibody Enhancer (Ready-to-use). <b>Reagent 1</b>	<ol style="list-style-type: none"> <li>a. Apply 2 drops (100 µL) or enough volume of MOUSE ANTIBODY ENHANCER to cover each section. Incubate in moist chamber for 10 min.</li> <li>b. Rinse with PBS for 2 min., 3 times.</li> </ol>	10
6. POLYMER-HRP for Rabbit (Ready-to-use) <b>Reagent 2</b>	<ol style="list-style-type: none"> <li>a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP to cover each section.</li> <li>b. Incubate in moist chamber for 10 min.</li> <li>c. Rinse with PBS for 2 min, 3 times.</li> </ol>	10
7. CHROMOGEN <b>Reagent 3A:</b> DAB Substrate <b>Reagent 3B:</b> DAB Chromogen	<ol style="list-style-type: none"> <li>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.</li> <li>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.</li> <li>c. Rinse with tap water for 1- 2 min.</li> </ol>	5
8. HEMATOXYLIN Supplied by user	<ol style="list-style-type: none"> <li>a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue completely and wait about 15-20 seconds.</li> <li>b. Rinse well under tap water for 1-2 minutes.</li> <li>c. Put slides in PBS until show blue color (about 30-60 seconds).</li> <li>d. Rinse well in distill or tap water</li> </ol>	15-20 <b>seconds</b>
9. Mounting medium: Supplied by user	<p>Follow the manufacture data sheet procedure for mounting. Recommended product:</p> <ol style="list-style-type: none"> <li>a. NeoBio Mount AQ (Cat# NB-23-00155-3, 18ml) for AEC, AP-Red, and AP-blue.</li> <li>b. NeoBio Mount Perm (Cat.# NB-23-00156, 18ml), for DAB</li> <li>c. 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</li> </ol>	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. Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum. We recommend NeoBlock, NB-23-00169-1 (500ml), NB-23-00169-2 (100ml), NB-23-00169-3 (18ml)

## 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 Plus Kit - HRP Mouse bulk kit (without chromogen)	NB-23-00049-2	110ml
PolyStain 2-Step Plus Kit - HRP Mouse DAB kit	NB-23-00049-3 / -4	18ml 6ml
PolyStain 2-Step Plus Kit - HRP Goat kit Bulk Kit (without chromogen)	NB-23-00051-2	110ml
PolyStain 2-Step Plus Kit - HRP Goat DAB kit	NB-23-00051-3 / -4	18ml 6ml
PolyStain 2-Step Plus Kit - HRP Broad Bulk Kit (Without chromogen, for Ms. & Rb.)	NB-23-00048-3	110ml
PolyStain 2-Step Plus Kit - HRP Broad DAB Kit (for mouse and rabbit antibody)	NB-23-00048-4 / -5	18ml 6ml
PolyStain 2-Step Plus Kit - HRP Rat-NM (No cross to Mouse ) Bulk kit (without chromogen)	NB-23-00052-1	110ml
PolyStain 2-Step Plus Kit - HRP Rat-NM (No cross to Mouse) DAB kit	NB-23-00052-2 / -3	18ml 6ml
PolyStain 2-Step Plus Kit - HRP Mouse-NR (No cross to Rat) Bulk kit (without chromogen)	NB-23-00053-1	110ml
PolyStain 2-Step Plus Kit - HRP Mouse-NR (No cross to Rat) DAB kit	NB-23-00053-2 / -3	18ml 6ml
DAB Kit (2-components)	NB-23-000148	12ml + 240ml
NeoBio Mount Perm	NB-23-000148	18ml