



PolyStain 2-Step Kit, HRP, Mouse, Rabbit & Goat

Polymer-HRP detection for mouse, rabbit, or goat primary antibodies using a biotin-free system

NB-23-00203-3 (110mL)

NB-23-00203-2 (18mL)

NB-23-00203-1 (6mL)



PolyStain 2-Step Kit, HRP, Mouse, Rabbit & Goat

NB-23-00203-1; NB-23-00203-2; NB-23-00203-3

Storage: 4-8°C

INTENDED USE:

PolyStain 2-Step Kit is a HRP polymer based detection system that can be used to detect mouse, rabbit, or goat primary antibodies for immunohistochemical screens of human tissues.

PolyStain 2-Step Kits 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:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	Polymer Helper (RTU)	6mL	18mL	110mL
Reagent 2	Polymer HRP anti-Mouse/Rabbit/Goat IgG (RTU)	6mL	18mL	110mL
Reagent 3A	DAB Substrate (RTU)	12mL	15mLx2	NA
Reagent 3B	DAB Chromogen (20x)	1.5mL	2mL	NA

RECOMMENDED PROTOCOL:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, the 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 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 as this will create uneven staining or background.
8. Serum blocking before primary antibody incubation for NeoBiotech's PolyStain -1, PolyStain -2, and PolyStain 2-Step is not required because all our antibody conjugates are absorbed to human serum.

Step/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 minutes. b. Rinse the slide using distilled water.	10 min.
2. HIER Pretreatment: (Optional)	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-BLOCKING (Optional) Not provided	a. Add 2 drops (100µL) or enough volume of 10% Normal Goat Serum (NB-23-00169) to completely cover the tissue section, Incubate for 10 min. b. Drain or blot off solution. DO NOT RINSE. c. See note 8 in Recommended Protocol.	10 min.
4. PRIMARY ANTIBODY Supplied by user	a. Apply 2 drops (100µL) 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 min

<p>5. Reagent 1</p> <p>Polymer Helper (RTU)</p>	<p>a. Apply 2 drops (100µL) or enough volume of Reagent 1 Polymer Helper to cover each section. Incubate in moist chamber for 15-30 min.</p> <p>(We recommend incubating the polymer helper up to 30mins for best sensitivity)</p> <p>b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.</p>	<p>15 - 30</p>
<p>6. Reagent 2</p> <p>Polymer HRP antiMouse/Rabbit/Goat IgG (RTU)</p>	<p>a. Apply 2 drops (100µL) or enough volume of Reagent 2 Polymer HRP antiMouse/Rabbit/Goat IgG to cover each section. Incubate in moist chamber for 15-30 min.</p> <p>(We recommend incubating the polymer helper up to 30mins for best sensitivity).</p> <p>b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.</p>	<p>15 – 30 min</p>
<p>7. CHROMOGEN</p> <p>Reagent 3A: DAB Substrate(RTU)</p> <p>Reagent 3B: DAB Chromogen(20x)</p>	<p>a. Add 1 drop of Reagent 3B into 1mL Reagent 3A. Mix well.</p> <p>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.</p> <p>c. Rinse well in distill or tap water.</p> <p>Note: Protect DAB working solution from light and use within 5 hours.</p>	<p>5 min</p>
<p>8. HEMATOXYLIN</p> <p>Supplied by user</p>	<p>a. Counterstain with 2 drops (100µL) or enough volume of Hematoxylin to cover tissue completely and wait about 20-60 seconds.</p> <p>b. Rinse well with running tap water for 1-2 minutes</p> <p>c. Put slides in PBS until show blue color (about 30-60 seconds).</p> <p>d. Rinse well in distilled or tap water</p>	<p>20 seconds</p>
<p>9. Mounting media:</p> <p>Supplied by user</p>	<p>We recommend an organic mounting media but you can use and aqueous mount. Follow the manufacture data sheet procedure for mounting. Recommended product:</p> <ol style="list-style-type: none"> 1. NeoMount AQ: Cat. No. NB-23-00155-3 (18mL), for Aqueous solution, use with AEC and AP-Red 2. NeoMount Perm: Cat. No. NB-23-00156 (18mL), for DAB,BCIP/NBT 3. NeoMount Universal: Cat. No. NB-23-00157-2 (18mL), or NB-23-00157-1 (100mL), Water Based, universal <p>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	Product	Catalog No	Size
PolyStain 2-Step Kit, AP, Mouse & Rabbit, no chromogen	NB-23-00047-1	110 ml	Fast Red Kit	NB-23-00142	12 Tab + 60mL
PolyStain 2-Step Kit, AP, Mouse & Rabbit, with Permanent Red	NB-23-00047-2	18 ml	AP-Red concentrated (40x) Kit for 2000 slides	NB-23-00143	8ml
	NB-23-00047-3	6ml			
PolyStain 2-Step Kit, HRP for AEC, Mouse & Rabbit, no chromogen	NB-23-00046-1	110ml	BCIP/NBT (RTU)	NB-23-00144-1	100ml
	NB-23-00046-2	60ml		NB-23-00144-2	18ml
PolyStain 2-Step Kit, HRP, Mouse & Rabbit, with AEC	NB-23-00046-3	18ml	NeoMount Universal (Water Based, universal) Kit	NB-23-00157-1	100ml
	NB-23-00046-4	6ml		NB-23-00157-2	18ml
PolyStain 2-Step Kit, AP, Mouse, Rabbit & Goat, no chromogen	NB-23-00200-3	110ml	Permanent-Red Kit	NB-23-00154-1	12 Tab + 60mL
PolyStain 2-Step Kit, AP, Mouse, Rabbit & Goat, with Permanent Red	NB-23-00200-1	18ml	Simplified HRP Mouse Kit (Concentrated, suggested 1:100-200)	NB-23-00154-2	18ml
	NB-23-00200-2	6ml			
AEC concentrated Kit (20x) for 2400 slides	NB-23-00140	12ml	DAB+ 2 components	NB-23-00148-1	12mL +240mL

PRECAUTIONS:

Handle all specimens as potentially infectious materials, wear gloves and appropriate personal protection equipment.

FOR RESEARCH USE ONLY



