



# PolyStain 2-Step Plus Kit, HRP for AEC, Mouse-NR

2-step Polymer-HRP detection system for mouse primary antibody, biotin-free

**NB-23-00065-1 (110 ml)**

**NB-23-00065-2 (18 ml)**

**NB-23-00065-3 (6 ml)**



## **PolyStain 2-Step Plus Kit, HRP for AEC, Mouse-NR**

NB-23-00065-1; NB-23-00065-2; NB-23-00065-3

**Storage: 4-8°C**

### **INTENDED USE:**

Detecting MOUSE primary antibody on RAT tissue is a very difficult task in research field due to background staining issues. PolyStain 2-Step Plus Kit, HRP for AEC, Mouse-NR (no cross react to rat) AEC Detection Kit is specially designed to solve the problem.

The secondary antibody is adsorbed to rat, rabbit and human serum proteins. This technology provides excellent specificity to detect mouse primary antibody (user supplied) on rat tissue. Besides rat tissue PolyStain 2-Step Plus, HRP for AEC, Mouse-NR kit also can be used on human tissue and rabbit tissue as well. PolyStain 2-Step Plus Kit, HRP for AEC, Mouse-NR Detection Kit is the 3rd generation of polymer detection system.

It uses mouse antibody 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-Plus detection system.

It is a biotin-free system, therefore, it 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.

It can be used for manual stain or autostainer. Staining conditions need to be optimized by user. PolyStain 2-Step Plus Kit, HRP for AEC, Mouse-NR 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:**

Catalog No.	Reagent	1	2	3A	3B	3C
	PolyStain 2-Step Plus Kit, HRP for AEC, Rat-NM	Mouse Antibody Enhancer (Ready-to-use)	Polymer HRP for Rat (Ready-to use)	AEC Substrate (20x)	AEC Chromogen (20x)	Hydrogen Peroxide (20x)
<b>NB-23-00065-1</b>	no chromogen	110 ml	110ml	Not included		
<b>NB-23-00065-2</b>	with AEC	18ml	18ml	2 ml	4 ml	2 ml
<b>NB-23-00065-3</b>	with AEC	6ml	6ml	1 ml	2 ml	1 ml

## **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 into as thin 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	Staining Procedures	Incubation Time (Min.)
1. PEROXIDASE BLOCKING REAGENT. Supplied by user	<ul style="list-style-type: none"> <li>a. Incubate slides in PEROXIDASE BLOCKING REAGENT (RTU 3% H<sub>2</sub>O<sub>2</sub> solution) for 10 minutes.</li> <li>b. Rinse the slide using distilled water.</li> </ul>	10 min
2. HIER PRETREATMENT:	<ul 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 3 times for 2 minutes each.</li> </ul>	
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	<ul 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> </ul>	30-60 min
5. Rat Antibody Enhancer (Ready-to-use). <b>Reagent 1</b>	<ul style="list-style-type: none"> <li>a. Apply 2 drops (100 µL) or enough volume of GOAT 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> </ul>	10 min
6. POLYMER-HRP for Mouse (Ready-to-use) <b>Reagent 2</b>	<ul style="list-style-type: none"> <li>a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for goat to cover each section. Incubate in moist chamber for 10 min</li> <li>b. Rinse with PBS for 2 min, 3 times.</li> </ul>	10min
7. CHROMOGEN <b>Reagent 3A:</b> AEC Substrate (20x) <b>Reagent 3B:</b> AEC Chromogen (20x) <b>Reagent 3C:</b> Hydrogen Peroxide (20x)	<ul style="list-style-type: none"> <li>a. Add 1 drop of Reagent <b>3A</b> into 1ml distilled water, mix well. Then add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent <b>3B</b> and 1 drop of Reagent <b>3C</b> in the diluted substrate buffer. Mix well. Protect from light and use within 1 hour.</li> <li>b. Apply 2 drops (100 µL) or enough of mixture completely cover tissue. Incubate for about 5 min. monitor the color development under microscope.</li> <li>c. Rinse with tap water for 1-2 min. <b>AEC is alcohol soluble, do not dehydrate!</b></li> </ul>	5 min
8. HEMATOXYLIN Supplied by user	<ul 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 <b>seconds</b>.</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 <b>seconds</b>).</li> <li>d. Rinse well in distill or tap water</li> </ul>	15-20 <b>seconds</b>

<b>9. Mounting medium:</b> Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: <ol style="list-style-type: none"><li>1. NeoMount AQ: Cat. No. NB-23-00155-3 (18ml), for Aqueous solution, use with AEC and AP-Red</li><li>2. NeoMount Universal: Cat.No. NB-23-00157-2 (18ml), or NB-23-00157-1 (100ml), Water Based, universal.</li></ol> Can be used with or without cover slip	Refer to insert
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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:**

Please wear gloves and take other necessary precautions

**FOR RESEARCH USE**

**RELATED PRODUCTS:**

Product	Catalog No	Size	Product	Catalog No	Size
PolyStain 2-Step Plus Kit, HRP for AEC, Mouse, no chromogen	NB-23-00061-1	110 ml	PolyStain 2-Step Plus Kit, HRP for AEC, Mouse & Rabbit, no chromogen	NB-23-00060-1	110 ml
PolyStain 2-Step Plus Kit, HRP, Mouse, with AEC	NB-23-00061-2 NB-23-00061-3	18 ml 6 ml	PolyStain 2-Step Plus Kit, HRP, Mouse & Rabbit, with AEC	NB-23-00060-2 NB-23-00060-3	18 ml 6 ml
PolyStain 2-Step Plus Kit, HRP for AEC, Goat, no chromogen	NB-23-00063-1	110 ml	PolyStain 2-Step Plus Kit, HRP for AEC, Rat-NM, no chromogen	NB-23-00064-1	110 ml
PolyStain 2-Step Plus Kit, HRP, Goat, with AEC	NB-23-00063-2 NB-23-00063-3	18 ml 6 ml	PolyStain 2-Step Plus Kit, HRP, Rat-NM, with AEC	NB-23-00064-2 NB-23-00064-3	18 ml 6 ml
PolyStain 2-Step Plus Kit, HRP for AEC, Rabbit, no chromogen	NB-23-00062-1	110 ml	AEC concentrated Kit (20x) for 2400 slides	NB-23-00140	12 ml
PolyStain 2-Step Plus Kit, HRP, Rabbit, with AEC	NB-23-00062-2 NB-23-00062-3	18 ml 6 ml	NeoMount Universal (Water Based, universal) Kit	NB-23-00157-2	18 ml

