



PolyStain 1-Step Kit - HRP  
Detection System for Rat  
Antibodies  
(for AEC)

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**NB-23-00038-1 (110ml, no chromogen)**

**NB-23-00038-2 (18ml, with AEC)**

**NB-23-00038-3 (6ml, with AEC)**

**PolyStain 1-Step Kit, Horseradish peroxidase Rat-NM  
(No cross react with Mouse) Detection System Kit for  
Rat Primary Antibodies (for AEC)**

**(Polymer-HRP detection system, biotin-free, Anti-rat primary antibody)**

**Ready-to-use One Step Polymer Detection System**

**Super clean when using rat antibody on mouse tissue**

<b>NB-23-00038-1</b>	<b>size : 60ml, no chromogen</b>
<b>NB-23-00038-2</b>	<b>size : 18ml, with AEC (good for 150 slides)</b>
<b>NB-23-00038-3</b>	<b>size : 6ml, with AEC (good for 50 slides)</b>

**Intended Use:**

Detecting RAT primary antibody on MOUSE tissue is a very difficult task in research field due to background issues. PolyStain 1-Step HRP Rat NM (no-mouse) AEC Detection kit is specially designed to solve the problem. This technology provides excellent specificity to detect rat primary antibody (user supplied) on mouse tissue. Specimen can be frozen or paraffin – embedded tissues, and freshly prepared monolayer cell smears. This detection system is super sensitive when use with AEC chromogen.

PolyStain 1-Step HRP Rat-NM AEC Detection kit is the ONE step polymer detection system that uses polymeric HRP-linked anti rat secondary antibody to directly detect rat primary antibody bound to the mouse tissue. The secondary antibody was adsorbed to mouse, rabbit and human serum proteins. Besides mouse tissue PolyStain 1-Step HRP Rat-NM AEC Detection kit also can be used on human tissue and rabbit tissue as well. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin<sup>1</sup>. It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2<sup>nd</sup> antibody, and then streptavidin-HRP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving.

If user needs most sensitive polymer detection system for rat primary antibody on mouse tissue, one may choose two-step polymer detection system, PolyStain 2-Step Plus HRP Rat-NM AEC kit (Cat# NB-23-00064-1, NB-23-00064-2, NB-23-00064-3).

**Kit Components:**

<b>Catalog No.</b>	<b>Reagent 1:</b> Polymer HRP-linked anti-rat IgG for AEC (Ready-to-use)	<b>Reagent 2:</b> 2A: substrate buffer (20x) 2B: Chromogen (20x) 2C: H <sub>2</sub> O <sub>2</sub> (20x)
NB-23-00038-1	110ml	Not provided
NB-23-00038-2	18ml	3ml of Reagent 2A 6ml of Reagent 2B 3ml of Reagent 2C
NB-23-00038-3	6ml	2ml of Reagent 2A 4ml of Reagent 2B 2ml of Reagent 2C

**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	Staining Procedure	Incubation Time (Min.)
1. Peroxidase Blocking Reagent Supplied by user	a. Incubate slides in peroxidase blocking reagent (Ready-to-use 3% H <sub>2</sub> O <sub>2</sub> solution) for 10 min. b. Rinse the slide using distilled water.	10
2. HIER Pretreatment: Refer to antibody data sheet.	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	a. Add 2 (100 µL) or more drops of 10% NeoBlock (NB-23-00169-1) to cover the tissue section and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE Drain.	10
4. Primary antibody: Supplied by user	<b>Notes:</b> Investigator needs to optimize dilution and incubation times 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. <b>Reagent 1:</b> HRP Polymer-anti-Rat IgG	a. Apply 2 (100 µL) or more drops of Polymer-HRP anti-Rat 2 <sup>nd</sup> antibody to cover tissue section and Incubate in moist chamber for 10-15 min. b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	15
6. <b>Reagents 2A, 2B and 2C:</b> AEC Chromogen (20x)	a. Add 1 drop of Reagent 2A, 1 drop or 2 drop (for high contrast) of Reagent 2B and 1 drop of Reagent 2C to 1 mL distilled or deionized water. Mix well. Protect from light and use within one hour. b. Apply 2 drops (100 µL) or enough volume of pre-mixed AEC Chromogen to completely cover tissue. Incubate for 5 min. to 10 min.. c. Rinse thoroughly with distill water	3-10
8. Hematoxylin: Supplied by user.	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 ½ - 1 min.) d. Rinse in distill water, then rinse well with tap water	20-30 seconds
9. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 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, for DAB & BCIP/NBT	Refer to insert

	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	
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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. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.

### Precautions:

Please wear gloves and take other necessary precautions.

### Remarks:

For research use only.

### Storage:

Store at 4°C.

### References:

1. Bisgaard K, Pluzed KP. Use of polymer conjugates in immunohistochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates. Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungary, October 20-25, 1996.
2. Shi ZR, Itzkowitz SH, Kim YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcinoma tissues. J Histochem Cytochem 36:317-322,

### Related products

Product	Catalog No.	Size
PolyStain 1-Step HRP Mouse kit for AEC	NB-23-00035-1	110ml
PolyStain 1-Step HRP Mouse kit 18ml, 6ml for AEC	NB-23-00035-2 / -3	18ml / 6ml
PolyStain 1-Step HRP Rabbit Bulk kit for AEC	NB-23-00036-1	110ml
PolyStain 1-Step HRP Rabbit 18ml, 6ml DAB Kit	NB-23-00036-2 / -3	18ml / 6ml
PolyStain 1-Step Goat Bulk kit for AEC	NB-23-00037-1	110ml
PolyStain 1-Step Goat 18ml, 6ml AEC Kit	NB-23-00037-2 / -3	18ml / 6ml
PolyStain 1-Step HRP Rat-NM Bulk kit for AEC (no x Mouse)	NB-23-00039-1	110ml
PolyStain 1-Step HRP Rat-NM 18ml, 6ml AEC Kit (no x Mouse)	NB-23-00039-2 / -3	18ml / 6ml
PolyStain 1-Step HRP Mouse/Rabbit Bulk kit for AEC	NB-23-00034-1	110ml
PolyStain 1-Step HRP HRP Mouse/Rabbit 18ml, 6ml AEC Kit	NB-23-00034-2 / -3	18ml / 6ml
AEC Kit	NB-23-00140	12ml
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