PolyStain 1-Step Kit, HRP Mouse-NR, AEC

Polymer-HRP detection system, biotin-free, Anti-mouse primary antibody

NB-23-00039-1 (110 ml, No chromogen)
NB-23-00039-2 (18 ml, with AEC)
NB-23-00039-3 (6 ml, with AEC)

PolyStain 2-Step Plus Kit, HRP f, Mouse-NR, AEC

NB-23-00039-1; NB-23-00039-2; NB-23-00039-3

INTENDED USE:

Storage: 4- 8ºC

Detecting MOUSE primary antibody on RAT tissue is a very difficult task in research field due to background issues. PolyStain 2-Step Plus Kit, HRP, Mouse-NR AEC Detection kit is specially designed to solve the problem. This technology provides excellent specificity to detect mouse primary antibody (user supplied) on rat 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 2-Step Plus Kit, HRP , Mouse-NR AEC Detection kit is the ONE step polymer detection system that uses polymeric HRP-linked anti mouse secondary antibody to directly detect mouse primary antibody bound to the rat tissue.

The secondary antibody was adsorbed to rat, rabbit and human serum proteins.

Besides rat tissue PolyStain 2-Step Plus Kit, HRP, Mouse-NR 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.

It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2nd 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 mouse primary antibody on rat tissue, one may choose two-step polymer detection system, PolyStain 2-Step Plus Kit, HRP, Mouse-NR, with AEC (Cat No. NB-23-00065-1, NB-23-00065-2, NB-23-00065-3).

KIT COMPONENTS:

	Reagent :	1	2A	2B	2C
Catalog No.	PolyStain 2-Step Plus Kit, HRP, Mouse-NR	Polymer HRP-linked anti-rabbit IgG for AEC (RTU)	substrate buffer (20x)	Chromogen (20x)	H_2O_2 (20x)
NB-23-00039-1	No Chromogen	110 ml	Not provided	Not provided	Not provided
NB-23-00039-2	With AEC	18 ml	3 ml	6 ml	3 ml
NB-23-00039-3	With AEC	6 ml	2 ml	4 ml	2 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 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.

Reagent	Staining Procedure	Incubation Time (Min.)
1. Peroxidase Blocking Reagent Supplied by user	a. Incubate slides in peroxidase blocking reagent (RTU 3% H_2O_2 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 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% Normal Goat Serum (**NB-23-00169**) to cover the tissue section and Incubate 10 min. b. Drain or blot off solution. **DO NOT RINSE.**	10

4. Primary antibody: Supplied by user	**Notes**: 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. Reagent 1: HRP Polymer-anti-rat (x mouse) RTU	a. Apply 2 (100 µL) or more drops of HRP Polymer-anti-Rat IgG 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	10-15
6. **Reagents 2A, 2B and 2C**: 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
7.Hematoxylin : Supplied by user.	a. Counterstain with 2 (100 µl) 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**
8. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 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 and BCIP/NBT 3. NeoMount Universal: Cat. No. **NB-23-00157-2** (18ml), or **NB-23-00157-1** (100ml), Water Based, universal Kit. Can be used with or without cover slip	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. 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.

RELATED PRODUCTS:

Product	Catalog No.	Size		Product	Catalog No.	Size
PolyStain 1-Step Kit, HRP for AEC, Mouse, no chromogen	NB-23-00035-1	110ml		* PolyStain 1-Step Kit, HRP, Mouse-NR, with AEC	NB-23-00039-2 NB-23-00039-3	18ml 6ml
PolyStain 1-Step Kit, HRP, Mouse, with AEC	NB-23-00035-2 NB-23-00034-3	18ml 6ml		PolyStain 1-Step Kit, HRP for AEC, Mouse & Rabbit, no chromogen	NB-23-00034-1 NB-23-00034-2	110ml 60 ml
PolyStain 1-Step Kit, HRP for AEC, Mouse, no chromogen	NB-23-00036-1	110ml		PolyStain 1-Step Kit, HRP for AEC, Mouse & Rabbit, no chromogen	NB-23-00034-3 NB-23-00034-2	18ml 6ml
PolyStain 1-Step Kit, HRP, Rabbit, with AEC	NB-23-00036-2 NB-23-00036-3	18ml 6ml		AEC concentrated Kit (20x)	NB-23-00140	12ml
PolyStain 1-Step Kit, HRP for AEC, Goat, no chromogen	NB-23-00037-1	110ml		NeoMount AQ (Aqueous solution, use with AEC and AP-Red)	NB-23-00155-3	18ml
PolyStain 1-Step Kit, HRP, Goat, with AEC	NB-23-00037-2 NB-23-00037-3	18ml 6ml		NeoMount Universal (Water Based, universal) Kit	NB-23-00157-1 NB-23-00157-2	100ml 18ml
* PolyStain 1-Step Kit, HRP for AEC, Mouse-NR, no chromogen	NB-23-00039-1	110ml				

*Polystain-1 HRP Mouse-NR kit does not cross react with mouse primary antibody

PRECAUTIOUS:

Please wear gloves and take other necessary precautions.

FOR RESEARCH USE ONLY

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