



PolyStain 1-Step Kit, HRP, Rabbit AEC

Polymer-HRP detection system for AEC staining, biotin-free, Anti-rabbit

NB-23-00036-1 (110 ml, No chromogen)

NB-23-00036-2 (18 ml, with AEC)

NB-23-00036-3 (6 ml, with AEC)

PolyStain 1-Step Kit, HRP Rabbit AEC

NB-23-00036-1; NB-23-00036-2; NB-2300036-3

Storage: 4- 8°C

INTENDED USE

PolyStain 1-Step Kit, HRP Rabbit AEC is designed to use with user supplied rabbit antibody to detect target antigen on human tissue or cell samples. 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 Kit, HRP Rabbit AEC is the ONE step polymer detection system that uses polymeric horseradish peroxidase (HRP) -linked goat anti Rabbit IgG to directly detect primary antibody that bound to the tissue. 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. 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.

KIT COMPONENTS

Reagent :		1	2A	2B	2C
Catalog No.	PolyStain 1-Step Kit, HRP Rabbit	Polymer HRP-linked anti- rabbit IgG for AEC (Ready-to-use)	substrate buffer (20x)	Chromogen (20x)	H ₂ O ₂ (20x)
NB-23-00036-1	No Chromogen	110 ml	Not provided	Not provided	Not provided
NB-23-00036-2	With AEC	18 ml	3 ml	6 ml	3 ml
NB-23-00036-3	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 ₂ O 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 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. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.	30 - 60
5. Reagent 1: HRP Polymer-anti- Rabbit (RTU)	a. Apply 2 (100 µL) or more drops of HRP Polymer-anti-Rabbit IgG to cover tissue section and Incubate in moist chamber for 10-15 min. b. Rinse with 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	5-10
7.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

<p>8. Mounting medium:</p> <p>Supplied by user</p>	<p>Follow the manufacture data sheet procedure for mounting.</p> <p>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 and BCIP/NBT 3. NeoMount Universal: Cat.No. NB-23-00157-2 (18ml), or NB-23-00157-1 (100ml), Water Based, universal Kit. <p>Can be used with or without cover slip</p>	<p>Refer to insert</p>
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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.

RELATED PRODUCTS:

Product	Catalog No.	Size	Product	Catalog No.	Size
PolyStain 1-Step Kit, HRP for AEC, Mouse & Rabbit, no chromogen	NB-23-00034-1	110ml	* PolyStain 1-Step Kit, HRP, Rat-NM, with AEC	NB-23-00038-2 NB-23-00038-3	18ml 6ml
PolyStain 1-Step Kit, HRP, Mouse & Rabbit, with AEC	NB-23-00034-3 NB-23-00034-4	18ml 6ml	** PolyStain 1-Step Kit, HRP for AEC, Mouse-NR, no chromogen	NB-23-00039-1	110ml
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-23-00035-3	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, Rat-NM, no chromogen	NB-23-00038-1	110ml			

* PolyStain 1-Step Plus Kit, HRP, Rat-NM does not cross react with mouse primary antibody

** PolyStain 1-Step Kit, HRP, Mouse-NR does not cross react with Rat primary antibody

PRECAUTIONS:

Please wear gloves and take other necessary precautions.

FOR RESEARCH USE ONLY