



PolyStain 2-Step Kit, HRP for AEC, Mouse & Rabbit, AEC

Polymer-HRP detection system, biotin-free, Anti-mouse/rabbit multivalent

NB-23-00046-1 (110 ml)

NB-23-00046-2 (60 ml)

NB-23-00046-3 (18 ml)

NB-23-00046-4 (6 ml)

PolyStain 2-Step Kit, HRP, Mouse & Rabbit, AEC
 NB-23-00046-1; NB-23-00046-2 ; NB-23-00046-3, NB-23-00046-4

Storage: 4- 8°C

INTENDED USE:

PolyStain 2-Step Kit, HRP, Mouse & Rabbit, AEC is the HRP polymer detection system that used for detect mouse and rabbit primary antibodies bond to tissue section. Polink-2 kit is the second generation polymer detection system that uses polymer helper and polymeric HRP-linked antibody conjugates to get consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies, especially on some nuclear-stained antibodies. 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. These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells.

This kit is super sensitive to AEC Chromogen.

KIT COMPONENTS:

	Reagent number:	1	2	3A	3B	3C
Catalog No	PolyStain 2-Step Kit, HRP, Mouse & Rabbit, AEC	Polymer Helper (RTU)	Polymer HRP-linked antimouse/rabbit antibody (RTU)	AEC Chromogen (20x)		
NB-23-00046-1	No chromogen	110ml	110ml	Not included		
NB-23-00046-3	With AEC	18ml	18ml	2 ml	4 ml	2 ml
NB-23-00046-4	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 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.

Reagent	Staining Procedures	Incubation Time (Min.)
1. PEROXIDASE BLOCKING REAGENT. Supplied by use	<ol style="list-style-type: none"> a. Incubate slides in PEROXIDASE BLOCKING REAGENT (RTU 3% H₂O₂ solution) for 10 minutes b. Rinse the slide using distilled water. 	10 min
2. HIER PRETREATMENT	<ol style="list-style-type: none"> 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 2 min., 3 times. 	
3. PRE-ANTIBODY BLOCKING (Optional)	<ol style="list-style-type: none"> a. Add 2 drops (100 µL) or enough volume of 10% Normal Goat serum to completely cover the tissue section, Incubate for 10 min. b. Drain or blot off solution. DO NOT RINSE 	10 min
4. PRIMARY ANTIBODY Supplied by user	<ol style="list-style-type: none"> 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. b. Rinse with PBS for 2 min., 3 times. 	30-60min
5. Reagent 1: POLYMER HELPER (RTU).	<ol style="list-style-type: none"> a. Apply 2 drops (100 µL) or enough volume of POLYMER HELPER to cover each section. Incubate in moist chamber for 15-20 min. b. Rinse with PBS for 2 min., 3 times. 	15-20min
6. Reagent 2 : POLYMER-HRP antimouse/rabbit IgG (RTU)	<ol style="list-style-type: none"> a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP to cover each section. Incubate in moist chamber for 15-20 min. b. Rinse with PBS for 2 min, 3 times. 	15-20min
7. Reagent 3A, 3B, 3C ; CHROMOGEN AEC (20x)	<ol style="list-style-type: none"> a. Add 1 drop of Reagent 3A, 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B and 1 drop of Reagent 3C 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 to completely cover tissue, of pre-mixed AEC to each section. Incubate for about 5 min. Monitor the color development under microscope c. Rinse well with distill or tap water (AEC is alcohol soluble; do not dehydrate.) 	5-10 min

8. HEMATOXYLIN Supplied by user	a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue completely and wait about 20-30 seconds. b. Rinse well under tap water for 1-2 minutes. c. Put slides in PBS until show blue color (about 30-60 seconds). d. Rinse well in distill or tap water	20-30 seconds
9. 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 Permanent mount for DAB,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 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, HRP for DAB, Mouse & Rabbit, no chromogen	NB-23-00045-6 NB-23-00045-7	110 ml 60 ml	Fast Red Kit	NB-23-00142	12 Tab + 60 mL
PolyStain 2-Step Kit, HRP, Mouse & Rabbit, with DAB	NB-23-00045-9 NB-23-00045-10	18 ml 6 ml	AP-Red concentrated (40x) Kit	NB-23-00143	8 mL
PolyStain 2-Step Kit, AP, Mouse & Rabbit, no chromogen	NB-23-00047-1	110 ml	BCIP/NBT (RTU)	NB-23-00144-1 NB-23-00144-2	100 ml 18 ml
PolyStain 2-Step Kit, AP, Mouse & Rabbit, with Permanent Red	NB-23-00047-2 NB-23-00047-3	18 ml 6 ml	NeoMount Universal (Water Based, universal) Kit	NB-23-00157-1 NB-23-00157-2	100 ml 18 ml
AEC concentrated Kit (20x)	NB-23-00140	12 ml	DAB+ 2 components	NB-23-00148-1	12 ml + 240 ml

PRECAUTION:

Please wear gloves and take other necessary precautions.

FOR RESEARCH USE ONLY