



PolyStain 2-Step Kit – AP Detection System for Mouse and Rabbit Antibodies

NB-23-00047-1 (110ml, no chromogen)

NB-23-00047-2 (18ml, with Permanent Red)

NB-23-00047-3 (60ml, with Permanent Red)

PolyStain 2-Step Kit – AP Detection System for Mouse and Rabbit Antibodies (Broad Spectrum)

***(Polymer-Alkaline Phosphatase detection system, biotin-free, Anti-mouse/rabbit multivalent)
Second Generation of Polymer Detection System***

*NB-23-00047-1 size : 110ml, no chromogen
NB-23-00047-2 size : 18ml, with Permanent Red
NB-23-00047-3 size : 60ml, with Permanent Red*

Intended Use:

PolyStain 2-Step Detection kit is an alkaline phosphatase (AP) polymer detection system that is used for detecting mouse and rabbit primary antibodies bound to tissue sections. PolyStain 2-Step kit is the second generation polymer detection system that uses polymer helper and polymeric AP-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.

Kit Components:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	Polymer Helper(RTU)	6mL	18mL	110mL
Reagent 2	Polymer AP anti-Mouse/Rabbit/Goat IgG(RTU)	6mL	18mL	110mL
Reagent 3A	Permanent Red Substrate (RTU)	7mL	18mL	NA
Reagent 3B	Permanent Red Activator (5x)	1.4mL	3.6mL	NA
Reagent 3C	Permanent Red Chromogen (100x)	70µL	180µL	NA

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. 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 for 2 minutes each time for 3 times..	Refer to vendor's data sheet
2. Pre-Block Solution Not included Recommend: NB-23-00169-1 / -2 / -3	a. Add 2 drops (100 µL) or enough volume of NeoBlock completely cover the tissue section, Incubate for 10 min. b. Drain or blot off solution. DO NOT RINSE	10
3. 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 for 2 minutes each time for 3 times.	30-60
4. Reagent 1: AP- POLYMER HELPER (Ready-to-use)	a. Apply 2 drops (100 µL) or enough volume of AP-POLYMER HELPER to cover each section. Incubate in moist chamber for 15-20 min. b. Rinse with PBS for 2 minutes each time for 3 times.	30
5. Reagents 2: AP-POLYMER anti-mouse/rabbit IgG (Ready-to-use)	a. Apply 2 drops (100 µL) or enough volume of AP- POLYMER to cover each section Incubate in moist chamber for 15-20 min. b. Rinse with PBS for 2 minutes each time for 3 times. c. Rinse with tap water.	15-20
6a. Chromogen (Supplied by user) or Reagent 3A, 3B, 3C: Reagent 3A: Permanent Red Substrate (RTU) Reagent 3B: Permanent Red Activator (5x) Reagent 3C: Permanent Red Chromogen (100x)	Recommended products: a. Fast-Red kit (Cat.# NB-23-00142) good for 600 slides b. AP-Red+ kit (Cat.# NB-23-00143) 40x good for approx. 3000 slides c. BCIP/NBT RTU kit (Cat.# NB-23-00144-1 / -2) Note: Shake Permanent Red Activator before adding into Permanent Red Substrate. a. Add 200µL of Reagent 3B into 1mL of Reagent 3A and mix well. Add 10µL of Reagent 3C into the mixture and mix well. (Note: For fewer slides, Add 100µL of Reagent 3B into 500µL of Reagent 3A and mix well. Add 5µL of Reagent 3C into the mixture and mix well.) b. Apply 2 drops (100µL) or enough volume of Permanent Red working solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development. c. Rinse well with distilled water.	10
8. Hematoxylin: Supplied by user.	a. Counterstain with Hematoxylin for 20-30 seconds. b. Rinse slides under tap water for 1-2 minutes b. Put slides in PBS until show blue color (about 60-90 seconds) c. Rinse well in distill or tap water.	20-30seconds
9. NeoBio Mount Universal: Cat.# NB-23-00157-2 (18ml), or NB-23- 00157-1 (100ml) Supplied by user	a. Apply 3 drops or enough volume of NeoBio Mount on the tissue. Must apply NeoBio Mount when tissue is wet. b. Rotate the slides to allow NeoBio Mount spread evenly to cover the tissue section, DO NOT cover slip on top of the NeoBio Mount. c. Place slides in an oven at 40-50°C for at least 30 minutes or leave it at room temperature until slides are thoroughly dried. Slow dry at room temperature will help to eliminate the air bubbles. Hardened NeoBio Mount forms an impervious permanent mount to organic solvents.	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. 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.

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 2-Step HRP Broad kit for DAB (w/o chromogen)	NB-23-00204-6	110ml
PolyStain 2-Step HRP Broad kit 18ml, 6ml (with DAB)	NB-23-00204-7 / -1	18ml / 6ml
PolyStain 2-Step HRP Broad Bulk kit for AEC (w/o AEC)	NB-23-00046-1 / -2	110ml / 60ml
PolyStain 2-Step HRP Broad AEC kit	NB-23-00046-3 / -4	18ml / 6ml
AEC kit (20x)	NB-23-00140	12ml
Fast Red Kit	NB-23-00142	12 tab + 60ml
AP-Red+ Kit (40x)	NB-23-00143	8ml
BCIP/NBT Kit	NB-23-00144-1 / -2	100ml / 18ml
DAB+ 2 components	NB-23-00148-1	12ml +240ml
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