



NeoStain Poly 2-Step
Plus Kit AP Rabbit

NB-23-00068-1

NB-23-00068-2

NB-23-00068-3

NeoStain Poly 2-Step Plus Kit AP Rabbit

#Cat: NB-23-00068-1 Size: 110mL (Bulk, w/o chromogen)
 #Cat: NB-23-00068-2 Size: 6mL (w/Permanent Red)
 #Cat: NB-23-00068-3 Size: 18mL (w/Permanent Red)

Storage: 2-8°C

Intended Use:

NeoStain Poly 2-Step Plus Kit AP Rabbit is the 3rd generation of polymer detection system. It uses rabbit antibody enhancer to help amplify the polymer enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of NeoStain Poly 2-Step Plus system. It is a biotin-free system; therefore, it overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. It can be used for manual stain or auto stainer. Staining conditions need to be optimized by user. NeoStain Poly 2-Step Plus Kit AP Detection System offers a wide choice for primary antibodies, including broad spectrum (for mouse and rabbit primary antibodies), mouse, rabbit, goat, and rat primary antibodies. Refer to Related Product section for details.

Kit components:

Component No	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	Rabbit Antibody Enhancer (RTU)	6mL	18mL	110mL
Reagent 2	Polymer AP anti-Rabbit (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 into as thin 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.
8. We recommend TBS-T to be used as the wash buffer to get the highest sensitivity and clean background. Phosphate in the PBS-T may inhibit the activity of the alkaline phosphatase. **Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. Neo Biotech sells 10xTBS-T for your convenience (NB-23-00201)**

Reagent	Staining Procedure	Staining Procedure
1. Hier Pretreatment:	<p>a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. Please check the data sheet of primary antibody</p> <p>b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T (See note 8 above); 3 times for 2 minutes each.</p>	
2. Primary Antibody Supplied by user	<p>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.</p> <p>b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p>	30-60min
3. Reagent 1 Rabbit Antibody Enhancer (RTU).	<p>a. Apply 2 drops (100 µL) or enough volume of Reagent 1 Rabbit Antibody Enhancer to cover each section. Incubate in moist chamber for 10 min.</p> <p>b. Wash with PBS/Tween (0.05%) 2 min., 3 times.</p>	10min
4. Reagent 2 Polymer AP anti-Rabbit (RTU)	<p>a. Apply 2 drops (100 µL) or enough volume of Reagent 2 Polymer AP anti-Rabbit to cover each section. Incubate in moist chamber for 10 min</p> <p>b. Wash with 1X TBS-T only; 3 times for 2 minutes each.</p>	10min
5. Reagent3A, 3B, 3C Reagent 3A: Permanent Red Substrate (RTU) Reagent 3B: Permanent Red Activator (5x) Reagent 3C: Permanent Red Chromogen (100x)	<p>a. Add 200µL of Reagent 3B (Activator) into 1mL of Reagent 3A (Substrate buffer) and mix well. Add 10µL of Reagent 3C(Chromogen) into the mixture and mix well. [Note: For fewer slides, add 100µL of Reagent 3B (Activator) into 500µL of Reagent 3A (Substrate buffer) and mix well. Add 5µL of Reagent 3C(Chromogen) into the mixture and mix well.]</p> <p>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.</p> <p>c. Rinse well with distilled water.</p>	10min
6. Hematoxylin: Supplied by user	<p>a. Counterstain with 2 (100µL) or more drops hematoxylin to cover tissue completely and wait about 20 seconds.</p> <p>b. Rinse well with tap water for 1-2 min.</p> <p>c. Put slides in PBS until the color turn blue (about ½ - 1 min.)</p> <p>d. Rinse in distill water, then rinse well with tap water</p>	20-30sec
7. Mounting medium: Supplied by user	<p>Follow the manufacture data sheet procedure for mounting. Recommended product:</p> <ol style="list-style-type: none"> NeoBio Mount AQ (Aqueous solution, use with AEC and AP-Red): Cat. No. NB-23-00155-3 (18ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) NeoBio Mount Universal (Water Based, universal) Kit: Cat.No. NB-23-00157-2(18ml), or NB-23-00157-1 (100ml), universal permanent mounting medium. 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. Permanent Red is insoluble in organic solvent and can be coversliped as well. however, the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance. Note: Please wipe off extra water and air-dry slides before dehydration and clear.
 - a. 1x 80% Ethanol 20 seconds;
 - b. 1x 95% Ethanol 20 seconds;
 - c. 3x 100% Ethanol 20 seconds each;
 - d. 1x 100% Xylene 20 seconds;
 - e. Add 1 drop of xylene based mountant (Cat. No. NeoBio Mount Perm (Permanent mount for DAB, BCIP/NBT), NB-23-00156) and coverslip. Press to push the air bubble out.

CAUTION: DO NOT dehydrate in xylene longer than 20 seconds! It will erase Permanent Red stain!

Related Products:

Product	Catalog No.	Size
NeoStain Poly 2-Step Plus Kit, AP, Mouse & Rabbit, no chromogen	NB-23-00066-2	110ml
NeoStain Poly 2-Step Plus Kit, AP, Mouse & Rabbit, with Permanent Red	NB-23-00066-3/ NB-23 00066-4	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, AP, Mouse, no chromogen	NB-23-00067-1	110ml
NeoStain Poly 2-Step Plus Kit, AP, Mouse, with Permanent Red	NB-23-00067-2/ NB-23-00068-3	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, AP, Rat-NM, no chromogen	NB-23-00070-1	110ml
NeoStain Poly 2-Step Plus Kit, AP, Rat-NM, with Permanent Red	NB-23-00070-2/ NB-23-00070-3	18ml / 6ml
NeoStain Poly 2-Step Plus Kit, AP, Mouse-NR, no chromogen	NB-23-00071-1	110ml
NeoStain Poly 2-Step Plus Kit, AP, Mouse-NR, with Permanent Red	NB-23-00071-2/ NB-23-00071-3	18ml / 6ml
AP-Red concentrated (40x) Kit for 3000 slides	NB-23-00143	8ml
BCIP/NBT (RTU)	NB-23-00144-1/ NB-23-00144-2	100ml / 18ml
NeoBio Mount AQ (Aqueous solution, use with AEC and AP-Red)	NB-23-00155-3	18ml
NeoBio Mount Universal (Water Based, universal) Kit	NB-23-00157-1/ NB-23-00157-2	100ml / 18ml