PolyStain 2-Step Plus Kit – AP Detection System for Rabbit Antibody

NB-23-00068-1 (110mL, no chromogen)
NB-23-00068-2 (18ml, with P.Red)
NB-23-00068-3 (6ml, with P.Red)

PolyStain 2-Step Plus Kit – Rabbit AP Detection System for Immunohistochemistry

(2-step Polymer-AP detection system, biotin-free)
Polymer Detection System with Super Sensitivity and Specificity

NB-23-00068-1 size : 110mL, no chromogen
NB-23-00068-2 size : 18ml, with Permanent Red (good for 180 slides)
NB-23-00068-3 size : 6ml, with Permanent Red (good for 50 slides)

Intended Use:

PolyStain 2-Step Plus AP Rabbit Detection Kit is the 3rd generation of polymer detection system. It uses rabbit antibody enhancer to help amplify the polymerenzyme 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 PolyStain 2-Step Plus detection 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 autostainer. Staining conditions need to be optimized by user.
PolyStain 2-Step Plus 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 for Rabbit (RTU)	6mL	18mL	110mL
Reagent 3A	Permanent Red Substrate (RTU)	7mL	18mL	NA
Reagent 3B	Permanent Red Activator (5x)	1.4mL	1.8mLx2	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. 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 inhibitor the activity of the alkaline phosphatase

Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. (We recommend 10xTBS-T NB-23-00201)

9. Serum blocking before primary antibody incubation for Neo Biotech's PolyStain 1-Step, PolyStain 2-Step, and PolyStain 2-Step Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent:

Reagent	Staining Procedure	Incubation Time (Min.)
1. Alkaline Phosphatase Blocking Reagent (Not provided)	a. Incubate slides in alkaline phosphatase blocking reagent. We recommend **Neo Biotech NeoPure Dual Enzyme Block** (NB-23-0193- 1 / -2). b. Rinse the slide using distilled water.	Refer to datasheet
2. HIER PRETREATMENT:	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-T containing 0.05% Tween-20 or 1X TBS-T (See note 8 above); 3 times for 2 minutes each.	Refer to datasheet
3. Pre-Block (optional) Not provided	a. Add 2 (100 µL) or more drops of NeoBlock (NB-23-0169-1 /-2 /-3) to cover the tissue section and Incubate 10 min b. Drain or blot off solution. DO NOT RINSE. c. See note 9 in Recommended Protocol.	10
4. PRIMARY ANTIBODY Supplied by user	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. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	30-60
5. Rabbit Antibody Enhancer (RTU) **Reagent 1**	a. Apply 2 drops (100µL) or enough volume of Reagent 1 Rabbit Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30 min. (We recommend incubating the antibody enhancer up to 30mins for best sensitivity) b. Wash with 1X TBS-T only; 3 times for 2 minutes each.	10-30
6. Polymer AP for Rabbit (RTU) **Reagent 2**	a. Apply 2 drops (100µL) or enough volume of POLYMER-AP for Rabbit to cover each section. Incubate in moist chamber for 10-30 min. b. (We recommend incubating the polymer up to 30mins for best sensitivity) Wash with 1xTBS-T 3 times for 2 minutes each.	10-30
7**. Reagent 3A, 3B, 3C** **Reagent 3A:** Permanent Red Substrate (RTU) **Reagent 3B:** Permanent Red Activator (5x) **Reagent 3C:** Permanent Red Chromogen (100x) To get maximum sensitivity of AP polymer, Repeat chromogen step	a. Shake Permanent Red Activator before adding into Permanent Red Substrate b. Add 200µL of Reagent 3B (Activator) into 1mL of Reagent 3A (Substrate) 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) and mix well. Add 5µL of Reagent 3C (Chromogen) into the mixture and mix well.] c. 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. To increase AP signal aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the GBI-Permanent Red working solution to completely cover the tissue for additional 5 to 10min. d. Rinse well with distilled water.	10

8. HEMATOXYLIN Supplied by user	a. Counterstain with 2 (100uL) 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.	15-20 **seconds**
9. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: a. NeoBio Mount AQ (Cat# NB-23-00155-3, 18ml) for AEC, AP-Red, and AP-blue. b. NeoBio Mount Universal (Cat.# 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 bevor dehydration and clear.

- 1x 80% Ethanol 20 seconds;
- 1x 95% Ethanol 20 seconds;
- 3x 100% Ethanol 20 seconds each;
- 1x 100% Xylene 20 seconds;

Add 1 drop of xylene based mountant (NB-23-00156 NeoBio Mount Perm) 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!

Precautious:

Please wear gloves, eye protection and take other necessary precautions. If any of the reagent come in contact with skin wash area completely with plenty of water and soap. If irritation develops seek medical attention.

Remarks:

For research use only.

Storage:

Store at 4°C.

Related products

Product	Catalog No.	Size
PolyStain 2-Step Plus Kit - AP Broad Bulk Kit (without chromogen)	NB-23-00066-2	110ml
PolyStain 2-Step Plus Kit - AP Broad kit	NB-23-00066-3 / -4	18ml / 6ml
PolyStain 2-Step Plus Kit - AP Goat Bulk Kit (without chromogen)	NB-23-00069-1	110ml
PolyStain 2-Step Plus Kit – AP Goat kit	NB-23-00069-2 / -3	18ml / 6ml

PolyStain 2-Step Plus Kit – AP Mouse Bulk Kit (without chromogen)	NB-23-00067-1	110ml
PolyStain 2-Step Plus Kit – AP Mouse Kit	NB-23-00067-2 / -3	18ml / 6ml
PolyStain 2-Step Plus Kit – AP Rat-NM Bulk kit (without chromogen) (no cross react to mouse)	NB-23-00070-1	110ml
PolyStain 2-Step Plus Kit - AP Rat-NM kit (no cross react to mouse)	NB-23-00070-2 / -3	18ml / 6ml
PolyStain 2-Step Plus Kit – AP Mouse-NR Bulk kit (without chromogen) (no cross react to rat)	NB-23-00071-1	110ml
PolyStain 2-Step Plus Kit - AP Mouse-NR Kit (no cross react to rat)	NB-23-00071-2 / -3	18ml / 6ml
Permanent Red Kit	NB-23-000154-1 /-2	18ml / 120ml
BCIP/NBT Kit	NB-23-000144-1 /-2	100ml / 18ml
NeoBio Mount AQ	NB-23-000155-2 /-3	100ml / 18ml
NeoBio Mount Perm	NB-23-000156	18ml
NeoBio Mount Universal	NB-23-00157-1 / -2	100ml / 18ml

References:

1. De Pasquale A, Paterlini P, Quaglino D.Immunochemical demonstration of different antigens in single cells in paraffin-embedded histological sections. Clin Lab Haematol. 1982;4(3):267-72.
2. Polak J. M and Van Noorden S. Introduction to Immunocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997