PolyStain 1-Step Kit – AP Detection System for Goat Antibodies (for Permanent Red)

NB-23-00044-1 (110ml, no chromogen)
NB-23-00044-2 (18ml, with Permanent Red)
NB-23-00044-3 (6ml, with Permanent Red)

PolyStain 1-Step Kit, AP Detection System Kit for Goat Antibodies (for Permanent Red)

(Polymer-AP detection system , biotin-free, Anti-goat)

Ready-to-use One Step Polymer Detection System

NB-23-00044-1 size : 110ml, no chromogen
NB-23-00044-2 size : 18ml, with chromogen
NB-23-00044-3 size : 6ml, with chromogen

Intended Use:

PolyStain 1-Step AP Goat Detection Kit is designed to use with user supplied rabbit antibody to detect targeted antigen on human tissue or cell samples. This kit has been tested in paraffin tissue. However, this kit can be used on frozen specimen and freshly prepared monolayer cell smears.
PolyStain 1-Step AP Goat Detection Kit is a one step polymer detection system that uses polymeric alkaline phosphatase (AP)-linked anti-goat 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[1]. It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2nd antibody, and then streptavidin-AP). 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 AP anti-Goat (RTU)	6mL	18mL	110mL
Reagent 2A	Permanent Red Substrate (RTU)	7mL	18mL	NA
Reagent 2B	Permanent Red Activator (5x)	1.4mL	3.6mL	NA
Reagent 2C	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.
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. (We recommend 10xTBS-T NB-23-00201)

9. Serum blocking before primary antibody incubation for Neo Biotech 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	Staining Procedure	Incubation Time (Min.)
1. Peroxidase Blocking Reagent Supplied by user	a. Incubate slides in peroxidese blocking reagent (Ready-to-use 3% H_2O_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-T containing 0.05% Tween-20 or 1X TBS-T (See note 8 above); 3 times for 2 minutes each.	Refer to vendor's data sheet
3. Pre-Block (Optional) Not provided	a. Add 2 (100 µL) or more drops of 10% Normal Donkey Serum (NB-23-00183-1 100ml / -2 18ml) 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	***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. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T (See note 8 above); 3 times for 2 minutes each.	30-60
5. **Reagent 1:** HRP Polymer AP anti-Goat IgG (Ready-to use)	a. Apply 2 (100 µL) or more drops of Polymer AP anti-Goat IgG to cover tissue section and Incubate in moist chamber for 15 min. **(We recommend incubating the polymer up to 30mins for best sensitivity.)** b. Wash with 1X TBS-T only; 3 times for 2 minutes each.	15-30
6. **Reagent 2A, 2B, 2C** Reagent 2A: Permanent Red Substrate (RTU) Reagent 2B: Permanent Red Activator (5x) Reagent 2C: Permanent Red Chromogen (100x) To get maximum sensitivity of AP polymer, Repeat chromogen	**Note**: Shake Permanent Red Activator before adding into Permanent Red Substrate. a. Add 200µL of **Reagent 2B** (Activator) into 1mL of **Reagent 2A** (Substrate buffer) and mix well. Add 10µL of **Reagent 2C** (Chromogen) into the mixture and mix well. (**Note**: For fewer slides, Add 100µL of Reagent 2B into 500µL of Reagent 2A and mix well. Add 5µL of Reagent 2C 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. To increase AP signal aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the Permanent Red working solution to completely cover the tissue for additional 5 to 10min. c. c. Rinse well with distilled water.	10
8. 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 ½-1min.) d. Rinse in distill water, then rinse well with tap water	20-30 **seconds**
9. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. NeoBio Mount AQ: Cat.# NB-00155-3 (18ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. NeoBio Mount Perm: Cat.# NB-23-00156 (18ml), for DAB and BCIP/NBT 3. 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 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.
5. 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 (NeoBio Mount Perm: Cat.# 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!

Precautious:
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 immunohitochemistry: 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, Hungry, October 20-25, 1996.
2. Shi ZR. Itzkowitz SH, Kim YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcimoma tissues. J Hitochem Cytochem 36:317-322,

Related products

Product	Catalog No.	Size
PolyStain 1-Step AP Mouse/Rabbit Bulk kit	NB-23-00040-1	110ml
PolyStain 1-Step AP Mouse/Rabbit kit 18ml, 6ml	NB-23-00040-2 / -3	18ml / 6ml
PolyStain 1-Step Mouse Bulk kit	NB-23-00041-1	110ml
PolyStain 1-Step Mouse Kit 18ml, 6ml	NB-23-00041-2 / -3	18ml / 6ml
PolyStain 1-Step Rabbit Bulk kit	NB-23-00042-1	110ml
PolyStain 1-Step Rabbit Kit 18ml	NB-23-00042-2	18ml
PolyStain 1-Step AP Rat-NM Bulk kit (no x Mouse)	NB-23-00045-1	110ml
PolyStain 1-Step AP Rat-NM 18ml, 6ml (no x Mouse)	NB-23-00045-2 / -3	18ml / 6ml
PolyStain 1-Step AP Mouse-NR Bulk kit (no x Rat)	NB-23-00043-1	110ml
PolyStain 1-Step AP Mouse-NR 18ml, 6ml AEC Kit (no x Rat)	NB-23-00043-2 / -3	18ml / 6ml
Fast Red Kit	NB-23-00142	12Tab + 60ml
BCIP/NBP Kit	NB-23-00144-1 /-2	100ml / 18ml
NeoBio Mount AQ (Aqueous)	NB-23-0015533	18ml
NeoBio Mount Universal	NB-23- 00157-1 / -2	100ml / 18ml