PolyStain DS Kit - for Rabbit and Rat antibody on Human & Mouse tissue

(BCIP/AEC)

NB-23-00126- 3(120 ml)

NB-23-00126- 2(36 ml)

NB-23-00126- 1(12 ml)

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PolyStain DS Kit - for Rabbit and Rat antibody on Human & Mouse tissue (BCIP/AEC)

NB-23-00126-1; NB-23-00126-2; NB-23-00126-3

Storage: 2-8ºC

INTENDED USE:

The PolyStain DS Kit is designed for use with user supplied rabbit and rat primary antibodies to detect two distinct antigens on human and mouse tissue or cell samples. PolyStain DS Kit can be developed for frozen or paraffin embedded tissue, or freshly prepared monolayer cell smears.

Double staining is a common method used in immunohistostaining, allowing for the detection of two distinct antigens in a single tissue. PolyStain DS Kit from NeoBiotech labs supplies the user with two polymer enzyme conjugates: HRP polymer anti-Rat IgG (minimal cross reaction to mouse) and AP polymer anti-Rabbit IgG with two distinct substrates/chromogens, BCIP/NBT and AEC. AEC chromogen reacts with the anti-Rat HRP polymer conjugate to produce a red color. BCIP/NBT reacts with anti-Rabbit AP polymer to produce the subsequent purple color.

PolyStain DS Kit is a non-biotin system avoiding the extra steps involved in blocking non-specific binding due to endogenous biotin.

KIT COMPONENTS:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Rat (No Ms) HRP(AEC) Polymer (RTU)	6mL	18mL	60mL
Reagent 2	Rabbit AP Polymer (RTU)	6mL	18mL	60mL
Reagent 3	BCIP/NBT (RTU)	15mL	18mL x 2	120mL
Reagent 4A	AEC Substrate (20x)	1mL	2mL	6mL
Reagent 4B	AEC Chromogen (20x)	2mL	4mL	12mL
Reagent 4C	Hydrogen Peroxide(20x)	1ml	2ml	6mL
Reagent 5	NeoMount Universal (RTU)	15mL	18mL x 2	120mL

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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 needs to be adhered to the slide tightly to avoid falling off.
3. Paraffin embedded sections must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be prepared as close to a monolayer as possible to obtain satisfactory results.
5. Three control slides are recommended for interpretation of results: positive, reagent (slides treated with Isotype control reagent), and negative control.
6. Proceed with IHC staining: **DO NOT** let specimen or tissue dry from this point on.
7. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting results.
8. Note: 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

Equipment or material needed but not provided:

1. Equipment and material for deparaffinization, such as fume absorbing hood, etc.
2. Heat source (microwave or hot plate) for HIER and antigen retrieval buffers.
3. Thermometer
4. Beaker
5. Timer
6. Wash buffer: 0.01 M PBS with 0.5% Tween20, pH7.4
7. Peroxidase and alkaline phosphatase blocking buffer
8. 100% ethanol
9. 100% Xylene
10. Hematoxylin

Reagent	Staining Procedure	Incubation Time (Min.)
1. Peroxidase and Alkaline Phosphatase Blocking Reagent **Not provided** Fast, easy and it will block endogenous alkaline phosphatase	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (NeoPure Dual Enzyme Block NB-23-00193 was Recommended) b. Rinse the slides using 2 changes of distilled water.	10 min.
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.	Up to 1 hour
3. Primary Antibody Mix: **one Rat and one Rabbit antibody** Supplied by user	**Note**: Investigator needs to optimize primary antibody titer prior to double staining. a. Apply 2 drops or enough volume of rat and rabbit primary antibody mixture to cover the tissue completely. Incubate in moist chamber for 30-60 min. Recommend 30min to shorten total protocol time. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	30-60 min
4. Mix Reagent 1& Reagent 2 **Reagent 1** Rat (No Ms) HRP(AEC) Polymer (RTU) **Reagent 2** Rabbit AP Polymer (RTU)	**Note**: Make sufficient polymer mixture by adding **Reagent 1** Rat (No Ms) HRP (AEC) Polymer and **Reagent 2** (Rabbit AP Polymer) at 1:1 ratio, mix well. a. Apply 1 to 2 drops (50-100µl) of the mixture to cover each section. b. Incubate in moist chamber for 30 min. c. Wash with 1X TBS-T only; 3 times for 2 minutes each. **Make enough mixture for the experiment. Do not make extra volume as mixture is not stable.**	30 min.
5. Reagents 3: BCIP/NBT Chromogen (RTU)	a. Apply 2 drops or enough volume of **Reagents 3** (BCIP/NBT Chromogen) to completely cover tissue. Incubate for 3-10 min. b. Rinse thoroughly with distilled water. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	3 - 10 min
6. Reagents 4A, 4B, 4C **4A:** AEC Substrate (20x)	a. Add 1 drop (50µl) of **Reagents 4A** and 1 drop or 2 drops (for higher sensitivity and contrast) **Reagents 4B** and 1 drop of **Reagents 4C** to 1mL distill water. Mix well. Keep away from	5 -10 min

4B: AEC Chromogen (20x) **4C:** Hydrogen Peroxide (20x)	light and use within 1 hour. b. Apply 2 drops (100µl) or enough volume of AEC chromogen working solution to completely cover the tissue. Incubate for 5-10 min, observe appropriate color development. c. Rinse well with distilled water. **(AEC is alcohol soluble; do not dehydrate.)**	
7.Counterstain Hematoxylin (Not provided)	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 5 to 10 seconds. **DO NOT** over stain with hematoxylin! b. Wash slides thoroughly with tap water for 1 minute. c. Put slides in PBS for 5-10 seconds to blue, **DO NOT** over blue. d. Wash slides well in distilled or tap water for 1 minute.	5 sec
8. Reagent 5: NeoMount Universal (RTU) **To coverslip see protocol note 3.**	**Note**: Investigator needs to optimize the primary antibodies dilution and incubation time prior to double staining. a. Apply 2 drops or enough volume of mouse primary antibody to cover the tissue completely. Mix well on the slide and 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 min

PROTOCOL NOTES:

1. The fixation, tissue slide thickness, antigen retrieval 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. NeoMount Universal is an aqueous-based mounting media for immunohistochemistry. It is used as the permanent mounting media for alcohol soluble chromogens such as AP-Red, AEC, and BCIP. NeoMount Universal does not use a coverslip. However, if you need to coverslip your tissue, after NeoMount Universal has dried, dip the slide in xylene (1 to 2 seconds), apply an organic mounting solution (such as NeoMount Perm, Cat# NB-23-00156), and place cover glass on the slide. Store slides after they have dried completely.

PRECAUTIONS:

Please wear gloves and take other necessary precautions

FOR RESEARCH USE

Work Sheet for NB-23-00126 Kit

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We designed these work sheets to help you track of each step. When staining fails these sheets help our technical support staff to pinpoint the problem. To insure that all steps are done properly, we recommend that the user fill in the actual time of their experimental step and any variation. Results will vary if time recommendations are not followed. RTU translates to ready to use.

- Used for tester to check "√ "each step during the experiment
- Steps follow after de-paraffinization
- Refer to insert for details of each step

Protocol Step	**NB-23-00126 Protocol**	**Experiment 1 Date:**	**Experiment 2 Date:**	**Experiment 3 Date:**	**Experiment 4 Date:**
Step 1	Peroxidase or Alkaline Phosphatase Block User supplied recommended NB-23-00193				
Step 2	HIER if needed				
Step 3	Rb 1°Ab & Rat 1°Ab mix (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** Rat (no Ms) HRP (AEC) Polymer & Rabbit AP Polymer require mixing (30 min.)				
Step 5	**Reagent 3** BCIP/NBT RTU (10min)				
Step 6	**Reagent 4A, Reagent 4B& Reagent 4C** AEC requires mixing! (10min)				
Step 7	Counter stain Hematoxylin User supplied				

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Step 8	**Reagent 5** NeoMount Universal (RTU) **To coverslip see protocol note 3.**				
Result	Stain pattern on controls are correct: Fill in Yes or No				

Testing result: