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

NB-23-00087- 3(120 ml)

NB-23-00087- 2(36 ml)

NB-23-00087- 1(12 ml)

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NB-23-00087-1; NB-23-00087-2; NB-23-00087-3

Storage: 4-8ºC

INTENDED USE:

The PolyStain DS Kit is designed to use with user supplied mouse and rabbit antibodies to detect two distinct antigens 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. Double staining is one of the most common methods used in immunohistochemistry to screen two distinct antigens in a single tissue NeoBioTech Labs the PolyStain DS Kit supplies user with two polymer enzyme conjugates; an HRP-Polymer anti-Mouse IgG and AP-Polymer anti-Rabbit IgG with reactive chromogens for each enzyme. The AEC chromogen (Red Brick color) is used with HRP-Polymer anti-Mouse IgG and BCIP/NBT (Purple/Blue color) is used with AP-Polymer anti-Rabbit IgG.

Simplified steps offer a much faster protocol as the enzyme conjugates are applied to the specimen as a mixture. Both the enzyme conjugated polymers and chromogens are optimized to give the strongest signal with no background. The PolyStain DS Kit is non-biotin system that avoids the need to block endogenous biotin causing non-specific binding

KIT COMPONENTS:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	HRP-Polymer(AEC) anti-Mouse IgG (RTU)	6mL	18mL	60 mL
Reagent 2	AP-Polymer anti-Rabbit IgG (RTU)	6mL	18mL	60mL
Reagent 3	BCIP/NBT (RTU)	12mL	18mL x 2	120 mL
Reagent 4A	AEC Substrate (20x)	1mL	2mL	6mL
Reagent 4B	AEC Chromogen (20x)	2mL	4mL	12 mL
Reagent 4C	Hydrogen Peroxide (20x)	1mL	2mL	6mL
Reagent 5	NeoMount Universal (100x)	12mL	18mLx2	120 mL

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 deparffinized 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. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
6. Proceed IHC staining: **DO NOT** let specimen or tissue dry from this point on.
7. 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.

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. We recommend **NeoPure Dual Enzyme Block NB-23-00193**. b. Rinse the slide using 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 7 above);** 3 times for 2 minutes each.	
3. Preblock (optional)	For paraffin section, Improved formula saves the need for a preblock step. For frozen tissue, preblock may or may not be required depending on fixative.	
4. Mouse antibody 1 and Rabbit antibody 2: **Supplied by user**	**Notes**: Investigator needs to optimize dilution and incubation times prior to double staining. a. Apply 2 drops or enough volume of both Primary Antibody 1 and Antibody 2 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.

5. Reagent 1 and 2: **Reagent 1 :** HRP Polymer anti-Mouse (RTU) **Reagent 2:** AP Polymer anti-Rabbit (RTU)	a. Apply 1drop (50µL) of **Reagent 1** HRP Polymer (AEC) anti-Mouse IgG and 1 drop of **Reagent 2** AP Polymer anti-Rabbit IgG to cover each section, mix well on the slide. Or you may prepare secondary antibodies cocktail in advance: 50µL **Reagent 1** HRP Polymer (AEC) anti-Mouse IgG plus 50µL **Reagent 2** AP Polymer anti-Rabbit IgG. b. Incubate in moist chamber for 30 min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each	30 min
6. Reagent 3: BCIP/NBT (RTU)	a. Apply 2 drops or enough volume of **Reagent 3** (BCIP/NBT) 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.	5-10min
7. Reagent 4A, 4B, 4C: **Reagent 4A:** AEC Substrate (20x) **Reagent 4B:** AEC Chromogen (20x) **Reagent 4C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µL) of **Reagent 4A** to 1mL distilled water. Mix well. Add 2 drops of **Reagent 4B** and 1 drop of **Reagent 4C** to diluted reagent 1. Mix well. Keep away from light and use within 1 hour. b. Apply 2 drops (100µL) or enough volume of pre-mixed AEC solution to completely cover the tissue. Incubate for 5-15min, observe appropriate color development. c. Rinse well with distilled water. **(AEC is alcohol soluble; do not dehydrate.)**	10 min
8. HEMATOXYLIN **Not provided**	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds. b. Rinse thoroughly with tap water for 2-3 min. c. Put slides in PBS until show blue color (about ½ - 1 min.) d. Rinse well in distilled water	
9. Reagent 5: NeoMount Universal (RTU)	a. Apply 2 drops (100µL) or enough volume Reagent 5 to cover tissue when tissue is wet. Rotate the slides to allow NeoMount Universal spread evenly. DO NOT coverslip. b. Place slides horizontally in an oven at 40-50°C for at least 30 minutes or leave it at room temperature until slides are thoroughly dried. Hardened NeoMount Universal forms an impervious polymer barrier to organic solvent. Do not use oil directly on the top of dried NeoMount Universal.	30 min in 40-50°C oven Or overnight at room temperature

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

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Work Sheet for NB-23-00087 Kit

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

NB-23-00087 Protocol is suitable when both mouse and rabbit primary antibodies need or do not need pre-treatment step.

Protocol Step	**NB-23-00087 Protocol Reagent / Time**	**Experiment 1 Date:**	**Experiment 2 Date:**	**Experiment 3 Date:**	**Experiment 4 Date:**
Step 1	Peroxidase or Alkaline Phosphatase Block User supplied				
Step 2 (Optional)	HIER if needed User supplied (up to 60 min)				
Step 3	Mouse 1°Ab & Rabbit 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** HRP-Polymer anti-Mouse IgG and AP-Polymer anti-Rabbit IgG require mixing (30min)				
Step 5	**Reagent 3A & Reagent 3B** DAB Requires mixing! (5min.)				
Step 6	**Reagent 4A, Reagent 4B& Reagent 4C** Note: Make fresh working solution and use				

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	immediately. Shake **Reagent 4B** well before adding into Reagent 4A. Permanent Red Requires mixing**! (10 min)** **To increase sensitivity, please repeat this step.**				
Step 7	Counter stain(10-15sec) User supplied				
Step 8	**Reagent 5** NeoMount Universal (RTU)				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

The result: