



# PolyStain DS Kit - for Mouse and Rat antibody on Mouse tissue

(BCIP/AEC)

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**NB-23-00123- 3(120 ml)**

**NB-23-00123- 2(36 ml)**

**NB-23-00123- 1(12 ml)**



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

NB-23-00123-1; NB-23-00123-2; NB-23-00123-3

### INTENDED USE:

**Storage: 2-8°C**

The PolyStain DS-MRt-Ms B Kit is designed to use with user supplied mouse and rat primary antibody to detect two distinct antigens on mouse tissue or cell samples. NB-23-00123 kits can be used on frozen specimens, paraffin-embedded tissues, or freshly prepared monolayer cell smears. NB-23-00123 kits is designed not to give background on most mouse strains. Double staining is one of most common methods used in immunohistostaining that allows detection of two distinct antigens in a single tissue. PolyStain DS-MRt-Ms B Kit from NeoBiotech Labs supplies two polymer enzyme conjugates: Mouse HRP (AEC) Polymer and Rat AP Polymer with two distinct substrates/chromogen, AEC (red color, use with the Mouse HRP Polymer) and BCIP/NBT Red (purple color, use with the Rat AP Polymer). A Primer step is used to increase specificity of antibody staining. This kit offers simplified steps that make for a quicker and easier protocol than that used in a sequential procedure. PolyStain DS-MRt-Ms B Kit is non-biotin system that avoids endogenous biotin non-specific binding.

### KIT COMPONENTS:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
<b>Reagent 1</b>	Rat AP Polymer (RTU)	6mL	18mL	60mL
<b>Reagent 2</b>	BCIP/NBT (RTU)	7mL	18mL	60mL
<b>Reagent 3A</b>	DS-MRt Block A (RTU)	6mL	18mL	60mL
<b>Reagent 3B</b>	DS-MRt Block B (RTU)	6mL	18mL	60mL
<b>Reagent 4</b>	Mouse Primer (RTU)	6mL	18mL	60mL
<b>Reagent 5</b>	Mouse HRP Polymer (RTU)	6mL	18mL	60mL
<b>Reagent 6A</b>	AEC Substrate (20x)	1mL	1mL	3mL
<b>Reagent 6B</b>	AEC Chromogen (20x)	2mL	2mL	6mL
<b>Reagent 6C</b>	Hydrogen Peroxide (20x)	1mL	1mL	3mL
<b>Reagent 7</b>	NeoMount Universal (RTU)	6mL	18mL	60mL

## **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. 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 with IHC staining: **DO NOT** let specimen or tissue dry from this point on.
7. **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.

1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6.

NeoBiotech sells 10xTBS-T for your convenience (NB-23-00201)

<b>Reagent</b>	<b>Staining Procedure</b>	<b>Incubation Time (Min.)</b>
<b>1. Peroxidase and alkaline phosphatase Blocking Reagent</b>  Supplied by user	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (NeoPure Dual Enzyme Block NB-23-00193 is Recommended) for 10 minutes. b. Rinse the slides using 2 changes of distilled water.	10 min.
<b>2. HIER Pretreatment:</b> Refer to antibody data sheet	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to antibody datasheet b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T ( <b>See note 7 above</b> ); 3 times for 2 minutes each	60 – 90 min.
<b>3. Rat primary antibody:</b> Supplied by user	<b>Note:</b> Investigator needs to optimize the primary antibodies dilution and incubation time prior to double staining. a. Apply 2 drops or enough volume of rat 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 <b>1X TBS-T</b> ; 3 times for 2 minutes each.	30-60 min
<b>4. Reagent 1:</b> Rat AP Polymer(RTU)	a. Add 2 drops (100µL) or enough volume of <b>Reagent 1</b> (Rat AP Polymer) to cover the tissue section and Incubate Room Temperature for 10- 15minutes. b. Wash with 1X TBS-T only; 3 times for 2 minutes each	15 min

<b>5. Reagents 2:</b> BCIP/NBT Chromogen (RTU)	a. Apply 2 drops or enough volume of <b>Reagents 2</b> (BCIP/NBT Chromogen) to completely cover tissue. Incubate for 10 min. b. Rinse thoroughly with distilled water. c. Wash with PBS-T containing 0.05% Tween-20 or <b>1X TBS-T</b> ; 3 times for 2 minutes each.	10 min
<b>6. Reagent 3A:</b> DS-MRt Block A (RTU)	a. Add 2 drops (100µL) or enough volume of <b>Reagent 3A</b> DS-MRt Block A to cover the tissue section and Incubate. b. Wash with PBS-T containing 0.05% Tween-20 or <b>1X TBS-T</b> ; 3 times for 2 minutes each.	30 min
<b>7. Reagent 3B:</b> DS-MRt Block B (RTU)	a. Add 2 drops (100µL) or enough volume of <b>Reagent 3B</b> DS-MRt Block B to cover the tissue section and Incubate. Do not exceed 5min. b. Wash with PBS-T containing 0.05% Tween-20 or <b>1X TBS-T</b> ; 3 times for 2 minutes each.	5 min.
<b>8. Mouse primary antibody:</b> Supplied by user	<b>Note:</b> 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 <b>1X TBS-T</b> ; 3 times for 2 minutes each.	30-60 min.
<b>9. Reagent 4:</b> Mouse Primer (RTU)	a. Add 2 drops (100µL) or enough volume of <b>Reagent 4</b> (Mouse Primer) to cover the tissue section and Incubate Room Temperature for 15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or <b>1X TBS-T</b> ; 3 times for 2 minutes each.	15 min.
<b>10. Reagent 5:</b> Mouse HRP(AEC) Polymer (RTU)	a. Add 2 drops (100µL) or enough volume of <b>Reagent 5</b> (Mouse HRP (AEC) Polymer) to cover the tissue section and incubate at Room Temperature for 15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or <b>1X TBS-T</b> ; 3 times for 2 minutes each.	30 min
<b>11. Reagent 6A, 6B, 6C:</b> <b>Reagent 6A:</b> AEC Substrate Buffer (20x) <b>Reagent 6B:</b> AEC Chromogen (20x) <b>Reagent 6C:</b> Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of <b>Reagent 6A</b> and 1 drop or 2 drops (for higher sensitivity and contrast) of <b>Reagent 6B</b> and 1 drop of <b>Reagent 6C</b> to 1ml distill water. 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 10 min, observe appropriate color development c. Rinse well with distilled water. (AEC is alcohol soluble; do not dehydrate.)	10 min

<b>12. Hematoxylin</b> 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 30 - 60sec) d. Rinse well in distilled water.	15 min.
<b>13. Reagent 7:</b> NeoMount Universal (RTU)	a. Apply 2 drops (100µL) or enough volume of <b>Reagent 7</b> (NeoMount Universal) to cover tissue when tissue is wet. Rotate the slides to allow NeoMount Universal spread evenly. 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.	

## 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 chromogen such as Permanent Red, 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-23-00123 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

Protocol Step	Protocol NB-23-00123	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase & Alkaline Phosphatase Block NB-23-00193 is recommend User supplied				
Step 2	HIER if needed Refer to datasheet				
Step 3	Rat 1°Ab (30-60 min.)				
Step 4	<b>Reagent 1</b> Rat AP Polymer (15 min) (Wash with TBS-T only)				
Step 5	<b>Reagent 2</b> BCIP/NBT (10min)				
Step 6	<b>Reagent 3A</b> DS-MRt Block A(RTU) 30min				
Step 7	<b>Reagent 3B</b> DS-MRt Block B(RTU) 5min				
Step 8	Mouse 1°Ab (30-60 min.)				
Step 9	<b>Reagent 4</b> Mouse Primer RTU (15 min)				
Step 10	<b>Reagent 5</b> Mouse HRP(AEC) Polymer (15 min)				

<b>Step 11</b>	<b>Reagent 6A,6B&amp;6C</b> AEC requires mixing! (10min)				
<b>Step 12</b>	Counter stain Hematoxylin User supplied				
<b>Step 13</b>	<b>Reagent 8</b> NeoMount Universal RTU Do not coverslip!				
<b>Result</b>	<b>Stain pattern on controls are correct: Fill in Yes or NO</b>				

**Testing result:**