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

(DAB/Permanent Red)

NB-23-00122- 3(120 ml)

NB-23-00122- 2(36 ml)

NB-23-00122- 1(12 ml)

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

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NB-23-00122-1; NB-23-00122-2; NB-23-00122-3

INTENDED USE:

Storage: 2-8ºC

The PolyStain DS-MRt-Ms A 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-23-00122 kits can be used on frozen specimens, paraffin–embedded tissues, or freshly prepared monolayer cell smears. NB-23-00122 kits is designed not to give background on most mouse strains however there may be some mouse strains especially when using frozen that require additional blocking; we recommend NeoBiotech's PureStain Mouse-on-Mouse Kit Blocking A & B solutions (NB-23-00076) to improve specificity of the mouse primary antibody on mouse tissue. 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 A Kit from NeoBiotech Labs-Inc. supplies two polymer enzyme conjugates: Mouse HRP Polymer and Rat AP Polymer with two distinct substrates/chromogen, DAB (brown color, use with the Mouse HRP Polymer) and Permanent Red (red 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 A Kit is non-biotin system that avoids endogenous biotin non-specific binding.

KIT COMPONENTS:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Rat AP Polymer (RTU)	6mL	18mL	60mL
Reagent 2A	Permanent Red Substrate (RTU)	7mL	18mL	60mL
Reagent 2B	Permanent Red Activator (5x)	1.4mL	3.6mL	12mL
Reagent 2C	Permanent Red Chromogen (100x)	70µL	180µL	0.6mL
Reagent 3A	DS-MRt Block A(RTU)	6mL	18mL	60mL
Reagent 3B	DS-MRt Block B(RTU)	6mL	18mL	60mL
Reagent 4	Mouse Primer (RTU)	6mL	18mL	60mL
Reagent 5	Mouse HRP Polymer (RTU)	6mL	18mL	60mL
Reagent 6A	DAB Substrate (RTU)	15mL	18mL	60mL
Reagent 6B	DAB Chromogen (20x)	1.5mL	2mL	3mL
Reagent 7	NeoMount Universal (RTU)	15mL	18mLx2	120mL

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 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)

Reagent	Staining Procedure	Incubation Time (Min.)
1. Peroxidase and alkaline phosphatase Blocking Reagent 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.
2. HIER Pretreatment: 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 **(See note 7 above);** 3 times for 2 minutes each	60 – 90 min.
3. Rat primary antibody: Supplied by user	**Note**: 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 **1X TBS-T**; 3 times for 2 minutes each.	30-60 min
4. Reagent 1: Rat AP Polymer(RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 1** (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

5. 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, Please repeat chromogen step)**	**Note**: Shake Permanent Red Activator before adding into Permanent Red Substrate. a. Add 200µL of **Reagent 2B** (Activator) into 1mL of **Reagent 2A** (Substrate) and mix well. Add 10µL of **Reagent 2C** (Chromogen) into the mixture and mix well. [Note: For fewer slides, Add 100µL of **Reagent 2B** (Activator) into 500µL of **Reagent 2A** (Substrate) and mix well. Add 5µL of **Reagent 2C** (Chromogen) 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. Rinse well with distilled water. d. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	10 min
6. Reagent 3A: DS-MRt Block A (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 3A** DS-MRt Block A to cover the tissue section and Incubate. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	30 min
7. Reagent 3B: DS-MRt Block B (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 3B** 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 1X TBS-T; 3 times for 2 minutes each	5 min.
8. Mouse primary antibody: Supplied by user	**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.
9. Reagent 4: Mouse Primer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 4** (Mouse Primer) to cover the tissue section and Incubate Room Temperature for 15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	15 min.
10. Reagent 5: Mouse HRP Polymer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 5** (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 **1X TBS-T**; 3 times for 2 minutes each.	15 min

11. Reagent 6A and 6B **Reagent 6A:** DAB Substrate (RTU) **Reagent 6B:** DAB Chromogen (20x)	a. Add 1 drop of **Reagent 6B** to 1mL of **Reagent 6A**. Mix well. Protect from light and use within 7 hours at 4 ºC. b. Apply 2 drops or enough volume of DAB Chromogen working solution to completely cover tissue. Incubate for 3-10 min. c. Rinse thoroughly with distilled water. DAB is a predicted carcinogen, wear gloves.	10 min
12. 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 30 - 60sec) d. Rinse well in distilled water.	
13. Reagent 7: NeoMount Universal (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 7** (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. **To coverslip see protocol note 3 below.**	

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. Permanent Red is insoluble in organic solvent and can be cover slipped 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 step.

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 (Cat. No. NeoMount Perm, 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!

PRECAUTIONS:

Please wear gloves and take other necessary precautions.

FOR RESEARCH USE

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Work Sheet for NB-23-00122 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-23-00122	**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	**Reagent 1** Rat AP Polymer (15 min) (Wash with TBS-T only)				
Step 5	**Reagent 2A, 2B&2C** Permanent Red requires mixing! (10min+10min)				
Step 6	Reagent 3A DS-MRt Block A(RTU) 30min				
Step 7	**Reagent 3B** DS-MRt Block B(RTU) 5min				
Step 8	Mouse 1°Ab (30-60 min.)				
Step 9	**Reagent 4** Mouse Primer RTU (15 min)				

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Step 10	**Reagent 5** Mouse HRP Polymer (15 min)				
Step 11	**Reagent 6A&6B** DAB requires mixing! (5min)				
Step 12	Counter stain Hematoxylin User supplied				
Step 13	**Reagent 8** NeoMount Universal (RTU) Do not coverslip!				
Result	**Stain pattern on controls are correct: Fill in Yes or NO**				

Testing result: