



PolyStain DS Kit - for 2 Mouse antibody on Rodent tissue

(BCIP/AEC)

NB-23-00102-1 (12ml)

NB-23-00102-2 (36ml)

NB-23-00102-3 (120ml)



PolyStain DS Kit - for 2 Mouse antibody on Rodent tissue (BCIP/AEC)

NB-23-00102-1; NB-23-00102-2; NB-23-00102-3

Storage: 2-8°C

INTENDED USE:

The PolyStain DS-MM Kit is designed to use with two user supplied mouse antibodies to detect two distinct antigens on mouse and rat tissue or cell samples. This kit has been tested on paraffin embedded tissue, which can be used for frozen or freshly prepared monolayer cell smears.

Double staining is a common method used in immunohistochemistry that allows for detection of two distinct antigens in a single tissue. This kit uses an HRP or AP polymer based technology combined with a proprietary blocking buffer system that achieves ultra-sensitivity with no background or cross reactivity. PolyStain DS-MM Kit from NeoBiotech labs supplies the user with primer system to enhance the two polymer enzyme conjugates anti-mouse IgG HRP polymer and anti-mouse IgG AP polymer with two distinct substrates/chromogen, GBIAEC and BCIP/NBT. BCIP/NBT reacts with anti-mouse IgG AP polymer conjugate to produce a purple color. AEC chromogen reacts with anti-mouse IgG HRP-polymer conjugate to produce a red color. PolyStain DS-MM Kit is a non-biotin system that avoids the extra steps involved in blocking non-specific binding due to endogenous biotin. Please read the protocol carefully and use the experimental record sheet to keep track of your progress throughout the protocol.

KIT COMPONENTS

Component No.	Content	12mL	36mL	120mL
Reagent 1	Mouse AP Polymer (RTU)	6mL	18mL	60mL
Reagent 2	BCIP/NBT (RTU)	7mL	18mL	60mL
Reagent 3	DAB Substrate(RTU)	15mL	18mLx2	120mL
Reagent 4A	DS-MM Blocker A (RTU)	6mL	18mL	60mL
Reagent 4B	DS-MM Blocker B (RTU)	6mL	18mL	60mL
Reagent 5	Mouse Primer (RTU)	6mL	18mL	60mL
Reagent 6	Mouse HRP(AEC) Polymer (RTU)	6mL	18mL	60mL
Reagent 7A	AEC Substrate (20x)	1mL	2mL	4mL
Reagent 7B	AEC Chromogen (20x)	2mL	4mL	8mL
Reagent 7C	Hydrogen Peroxide (20x)	1mL	2mL	4mL
Reagent 8	NeoMount Universal (RTU)	7mL	18mL	70mL

Protocol Notes:

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 Not provided Fast, easy and it will block endogenous alkaline phosphatase	<ol style="list-style-type: none"> Incubate slides in PEROXIDASE BLOCKING REAGENT we recommend NeoPure Dual Enzyme Block NB-23-00193 Rinse the slide using distilled water. 	10 min.
2. HIER Pretreatment: Refer to antibody data sheet.	<ol style="list-style-type: none"> Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to antibody datasheet. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T (See note 7 above); 3 times for 2 minutes each. No background issues go to step 5; if background an issue go to step 3.	60 - 90 min
3. Optional: Block step 1 Reagent Normal Rat serum blocking buffer NB-23-00190 free 1mL sample	Provided in this kit is a 1 ml sample of Normal Rat serum blocking buffer NB-23-00190 this block has been a staple in many labs screening mouse primary antibodies on mouse tissue. <ol style="list-style-type: none"> Apply 2 drops or enough volume of Normal Rat serum blocking buffer NB-23-00190 to cover the tissue completely. Incubate in moist chamber for 30min. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each. 	30 min.

<p>4. Optional: Block step 2 Reagent Normal Rat serum blocking buffer NB-23-00190 free 1mL sample</p>	<p>Use this block only if Reagent NB-23-00190 was used in step 3.</p> <ol style="list-style-type: none"> Apply 2 drops or enough volume of rat blocking buffer (Reagent NB-23-00190) to cover the tissue completely. Incubate in moist chamber for 5min. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each. 	<p>5 min</p>
<p>5. Ms Primary Antibody 1: Supplied by user</p>	<p>Notes: Investigator needs to optimize dilution and incubation times prior to double staining. Should use as dilute as possible to prevent cross reaction.</p> <ol style="list-style-type: none"> Apply 2 drops or enough volume of mouse primary antibody 1 to cover the tissue completely. Incubate in moist chamber for 30-60 min. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each. 	<p>30 -60 min.</p>
<p>6. Reagent 1: Mouse AP Polymer</p>	<ol style="list-style-type: none"> Apply 1-2 drops Reagent 1 (Mouse AP Polymer) or enough to cover each section. Incubate in moist chamber for 15 min. Wash with PBS containing 0.05% Tween-20 for 3 times for 2 min each. Wash with 1X TBS-T only; 3 times for 2 minutes each. 	<p>15 min.</p>
<p>7. Reagent 2: BCIP/NBT (RTU)</p>	<ol style="list-style-type: none"> Apply 2 drops or enough volume of Reagent 2 (BCIP/NBT) to completely cover tissue. Incubate for 5-10 min. Rinse thoroughly with distilled water. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each. 	<p>5 – 10 min.</p>
<p>8. Reagent 3: Antibody Blocker (40x) (Optional) Must test if antibody/antigen interaction is heat sensitive. Please skip this step if antigen retrieval is used for 2nd Ms Primary Antibody after step 7.</p>	<p>Note: This step will block antibodies of previous step so no cross reaction will occur at end of protocol.</p> <ol style="list-style-type: none"> Use hot plate or water bath to heat diluted Reagent 3 to 1x solution (1 part of Antibody Blocker in 39 parts of distilled water) to 80-95°C. Make enough volume to cover the tissue in beaker. For paraffin embedded tissue, put slides in heated Antibody Blocker for 10 minutes at 95°-100°C. For frozen embedded tissue, put slides in heated Antibody Blocker for 10 minutes at 80°C. Cool slides to 55°C. Rinse slides in multiple changes of distilled water. 	<p>10 min</p>

<p>9. Reagent 4A: DS-MM Blocker A (RTU)</p>	<p>a. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p> <p>b. Apply 2 drops or enough volume of Reagent 4A (DS-MM Blocker A) to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 30 min.</p> <p>c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p>	<p>30 min.</p>
<p>10. Reagent 4B: DS-MM Blocker B (RTU)</p>	<p>a. Apply 2 drops or enough volume of Reagent 4B (DS-MM Blocker B) to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 5 min.</p> <p>b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p>	<p>5 min.</p>
<p>11. Ms Primary Antibody 2: Supplied by user</p>	<p>Notes: Investigator needs to optimize dilution and incubation times prior to double staining.</p> <p>a. Apply 2 drops or enough volume of mouse primary antibody 2 to cover the tissue completely.</p> <p>b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p>	<p>30-60 min</p>
<p>12. Reagent 5: Mouse Primer (RTU)</p>	<p>a. Apply 1-2 drops Reagent 5 (Mouse Primer) or enough to cover each section.</p> <p>b. Incubate in moist chamber for 10 min.</p> <p>c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p>	<p>15 min.</p>
<p>13. Reagent 6: Mouse HRP(AEC) Polymer (RTU)</p>	<p>a. Apply 1-2 drops Reagent 6 Mouse HRP (AEC) Polymer to cover each section.</p> <p>b. Incubate in moist chamber for 10 min.</p> <p>c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.</p>	<p>30 min</p>
<p>14. Reagent 7A, 7B, 7C: Reagent 7A: AEC Substrate (20x) Reagent 7B: AEC Chromogen (20x) Reagent 7C: Hydrogen Peroxide (20x)</p>	<p>a. Add 1 drop (50µL) of Reagent 7A to 1mL distilled water. Mix well Add 2 drops of Reagent 7B and 1 drop of Reagent 7C to diluted reagent 1. Mix well. Keep away from light and use within 1 hour.</p> <p>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.</p> <p>c. Rinse well with distilled water. (AEC is alcohol soluble; do not dehydrate.)</p>	<p>15 min</p>
<p>15. HEMATOXYLIN Not provided</p>	<p>a. Counterstain with 2 drops (100µL) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds.</p> <p>b. Rinse thoroughly with tap water for 2-3 min</p> <p>c. Put slides in PBS or Tris pH 7.4 to 8.4 until blue color appears.</p> <p>d. Rinse well in distilled water.</p>	<p>5 min.</p>

16. Reagent 8: NeoMount Universal (RTU)	a. Apply 2 drops (100µL) or enough volume of Reagent 8 (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.	30 min. 50°C oven or overnight at room temperature
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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.

PRECAUTION:

Please wear gloves and take other necessary precautions.

For research use only

Work Sheet for NB-23-00102 Kit

We designed work sheet to help you track each step. You may use this sheet for our technical support staff to review if needed. To insure that all steps are done properly, we recommend that the user fill in the actual time of their experimental step. 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-23-00102 Protocol-1 is suitable for: 1) Both mouse primary antibodies need pre-treatment; 2) One mouse primary antibody needs pre-treatment and the other one is not sensitive to pretreatment.

	Main Protocol Step	NB-23-00102 Protocol-1	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
1	Step 1	Peroxidase and phosphatase Block User supplied NB-23-00193 is recommended.				
2	Step 2 Optional	HIER if needed User supplied (up to 60 min)				
3	Step 3 Optional	NB-23-00190 Normal Rat serum blocking buffer (30min)				
4	Step 4 Optional	NB-23-00190 Normal Rat serum blocking buffer (5 min)				
5	Step 5	Ms 1°Ab #1 User supplied (30-60 min)				
6	Step 6	Reagent 1 Mouse AP Polymer RTU (15 min) Wash with 1xTBS-T only.				
7	Step 7	Reagent 2 BCIP/NBT RTU (10 min)				
8	Step 8	Reagent 3 Antibody Blocker(40x) (10 min)				

9	Step 9	Reagent 4A: DS-MM Blocker A RTU (30 min)				
10	Step 10	Reagent 4B: DS-MM Blocker B RTU (5 min)				
11	Step 11	Ms 1°Ab #2 User supplied (30-60 min)				
12	Step 12	Reagent 5: Mouse Primer RTU (5 min)				
13	Step 13	Reagent 6 Ms HRP Polymer RTU (15 min)				
14	Step 14	Reagent 7A, 7B & 7C AEC requires mixing (10min)				
15	Step 15	Counter stain Hematoxylin User supplied				
16	Step 16	Reagent 8 NeoMount Universal (RTU) Do not coverslip!				
	Result	Stain pattern on controls are correct: Fill in Yes or NO				

NB-23-00102 Protocol-2 is suitable for one mouse primary antibody needs pre-treatment, the other mouse primary antibody is sensitive to pretreatment.

	Main Protocol Step	NB-23-00102 Protocol-2	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
1	Step 1	Peroxidase and phosphatase Block User supplied NB-23-00193 is recommended.				
2	Step 3 Optional	NB-23-00190 Normal Rat serum blocking buffer (30min)				
3	Step 4 Optional	NB-23-00190 Normal Rat serum blocking buffer (5 min)				
4	Step 5	Ms 1°Ab #1 User supplied (30-60 min) 1°Ab is sensitive to pre-treatment				
5	Step 6	Reagent 1 Mouse AP Polymer RTU (15 min) Wash with 1xTBS-T only				
6	Step 7	Reagent 2 BCIP/NBT RTU (10 min)				
7	Step 8	Reagent 3A & 3B DAB Requires mixing! (5 min)				
8	Step 2	HIER (10-15 min) Cool down (45-60 min) User supplied Skip antibody blocker step 8 if HIER is done since they will achieve same goal				
9	Step 9	Reagent 4A: DS-MM Blocker A RTU (30 min)				
10	Step 10	Reagent 4B: DS-MM Blocker B RTU (5 min)				
11	Step 11	Ms 1°Ab #1 User supplied (30-60 min) 1°Ab is not sensitive to pre-treatment				

12	Step 12	Reagent 5: Mouse Primer RTU (5 min)				
13	Step 13	Reagent 6 Ms HRP Polymer RTU (15 min)				
14	Step 14	Reagent 7A, 7B & 7C AEC requires mixing (10min)				
15	Step 15	Counter stain Hematoxylin User supplied				
16	Step 16	Reagent 8 NeoMount Universal RTU Do not coverslip!				
	Result	Stain pattern on controls are correct: Fill in Yes or NO				

