



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

(DAB/Permanent Red)

NB-23-00125- 3(120 ml)

NB-23-00125- 2(36 ml)

NB-23-00125- 1(12 ml)



**PolyStain DS Kit - for Rabbit and Rat antibody on Human & Mouse tissue
(DAB/Permanent Red)**

NB-23-00125-1; NB-23-00125-2; NB-23-00125-3

Storage: 2-8°C

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. This kit has been tested on paraffin embedded tissue. However, this kit can be used to stain frozen specimen and/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, DAB and Permanent Red. DAB chromogen reacts with the HRP polymer anti-Rat conjugate to produce a brown color. Permanent Red reacts with AP polymer anti-Rabbit conjugate to produce the subsequent red color. PolyStain DS Kit 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 Polymer(RTU)	6mL	18mL	60mL
Reagent 2	Rabbit AP Polymer(RTU)	6mL	18mL	60mL
Reagent 3A	DAB Substrate (RTU)	15mL	18mL x 2	120mL
Reagent 3B	DAB Chromogen (20x)	1.5mL	2mL	6mL
Reagent 4A	Permanent Red Substrate (RTU)	15mL	18mL x 2	120mL
Reagent 4B	Permanent Red Activator (5x)	3mL	7.2mL	12mL x 2
Reagent 4C	Permanent Red Chromogen (100x)	150µL	360µL	1.2mL
Reagent 5	NeoMount Universal	15mL	18mL x2	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 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.

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 suggested by vendor. b. Wash with PBS/0.05% Tween-20 for 2 minutes, 3 times	UP to 1 hour
3. Primary Antibody Mix: one Rat and one Rabbit antibody Supplied by user	Note: Investigator needs to optimize dilution 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/0.05% Tween20 for 2 minutes, 3 times.	30 – 60 min
4. Polymer mixture: Reagent 1: Rat(No Ms) HRP Polymer (RTU)	Note: Make sufficient polymer mixture by adding Reagent 1 Rat (No Ms) HRP 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.	30 min

<p>Reagent 2: Rabbit AP Polymer (RTU)</p>	<p>c. Wash with PBS/ 0.05% Tween20 for 2 minutes, 3 times. Make enough mixture for the experiment. Do not make extra volume as mixture may not be stable.</p>	
<p>5. Reagent 3A & Reagent 3B:</p> <p>Reagent 3A: DAB Substrate(RTU)</p> <p>Reagent 3B: DAB Chromogen (20x)</p>	<p>Note: Make enough DAB mix by adding 1 drop of Reagent 3B (DAB Chromogen) in 1mL of Reagent 3A (DAB Substrate). Mix well. Use within 7 hours.</p> <p>a. Apply 1 to 2 drops (50-100µL) of your DAB working solution to cover the tissue completely.</p> <p>b. Incubate for 5min.</p> <p>c. Rinse slides in multiple changes of distilled water 3 times, 2min each time or under running tap water for 1minute.</p>	5 min
<p>6. Reagent 4A, 4B, 4C</p> <p>Reagent 4A: Permanent Red Substrate (RTU)</p> <p>Reagent 4B: Permanent Red Activator (5x)</p> <p>Reagent 4C: Permanent Red Chromogen (100x)</p>	<p>a. Add 200µL of Reagent 4B (Activator) into 1mL of Reagent 4A (Substrate buffer) and mix well. Add 10µL of Reagent 4C (Chromogen) into the mixture and mix well. (Note: For fewer slides, Add 100µL of Reagent 4B (Activator) into 500µL of Reagent 4A (Substrate buffer) and mix well. Add 5µL of Reagent 4C (Chromogen) into the mixture and mix well.)</p> <p>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.</p> <p>c. Rinse well with distilled water.</p>	10 min
<p>7. HEMATOXYLIN</p> <p>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 until show blue color (about 30 - 60 sec.)</p> <p>d. Rinse well in distilled water.</p>	
<p>8. Reagent 5:</p> <p>NeoMount Universal (RTU)</p>	<p>Apply 2 drops (100µL) or enough volume of Reagent 5 NeoMount Universal to cover tissue when tissue is wet. Rotate the slides to allow NeoMount Universal spread evenly.</p>	

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 coverslipped 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.

- 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:

DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

FOR RESEARCH USE ONLY

Work Sheet for NB-23-00125 Kit

We designed this work sheet to help you track of each step. We recommend you use this sheet to record the actual time of each step conducted as it will be helpful for questions with our technical support. 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-23-00125 Protocol is suitable when both Rabbit and rat primary antibodies need or do not need pre-treatment step

Protocol Step	Protocol NB-23-00125	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase or Alkaline Phosphatase Block NB-23-00193 is recommended. supplied				
Step 2	HIER if needed				
Step 3	Rb 1°Ab & Rat 1°Ab mix (30-60 min.)				
Step 4 Optional	Reagent 1 & Reagent 2 Rat HRP Polymer & Rabbit AP Polymer require mixing (30 min.)				
Step 5	Reagent 3A & Reagent 3B DAB requires mixing (5min)				
Step 6	Reagent 4A, Reagent 4B & Reagent 4C Permanent Red requires mixing (10min)				

Step 7	Counter stain User supplied				
Step 8	Reagent 5 NeoMount Universal RTU				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Testing result: