NeoStain Poly DS Kit - for 2Rabbit antibody on Human/Rodent tissue

NB-23-00105

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#Cat : NB-23-00105-3 **Size: 120 ml**
#Cat : NB-23-00105-2 **Size: 36 ml**
#Cat : NB-23-00105-1 **Size: 12 ml**

Storage: 2-8ºC

Intended use:

NeoStain Poly DS Kit is designed to use with user supplied two 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 onfrozen specimen and freshly prepared monolayer cell smears. Double staining is one of most common methods used in immunohistostaining that allow revealing two distinct antigens in a single tissue. NeoStain Poly DS Kit from NeoBiotech Labs supplies two polymer enzyme conjugates: HRP polymer anti-Rabbit IgG andAP polymer anti-Rabbit IgG with two distinct substrates/chromogens, DAB (brown color, use with HRP polymer anti-Rabbit IgG) and Permanent Red (red color, use with AP polymer anti-Rabbit IgG). When the AP Polymer anti-Rabbit antigen is present only the Permanent Red will be present or when HRP Polymer anti-rabbit antigen is present only the DAB (brown) will be present. NeoStain Poly DS Kit is non-biotin system that avoids endogenous biotin nonspecific binding.

Kit components:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Rabbit HRP Polymer (RTU)	6ml	18ml	60ml
Reagent 2A	DAB Substrate (RTU)	12ml	15ml x 2	70ml
Reagent 2B	DAB Chromogen (20X)	1.5ml	2ml	3.5ml
Reagent 3A	DS-RR-Blocker A (RTU)	6ml	18ml	60ml
Reagent 3B	DS-RR-Blocker B (RTU)	6ml	18ml	60ml
Reagent 4	Rabbit AP Polymer (RTU)	6ml	18ml	60ml
Reagent 5A	Permanent Red Substrate (RTU)	15ml	18ml x 2	70ml
Reagent 5B	Permanent Red Activator (5x)	3ml	7.2ml	14ml
Reagent 5C	Permanent Red Chromogen (100x)	150µl	360µl	0.7ml
Reagent 6	NeoMount Universal (RTU)	7ml	18ml	70ml

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Recommended protocol:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, user needs tosupply 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 ofethanol 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 cleanbackground. Phosphate in the PBS-T may inhibitor the activity of the alkaline phosphatase.

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.	
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. (Preblock catalogue No.NB-23-00169 was Recommended.)	
4. Rabbit Antibody 1: Supplied by user	**Notes**: Investigator needs to optimize dilution and incubation times priorto double staining. a. Apply 2 drops or enough volume of rabbit primary antibody 1 to coverthe tissue completely. 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: Rabbit HRP polymer(RTU)	a. Apply 1drop (50µL) of Reagent 1 (Rabbit HRP) polymer to cover eachsection. b. Incubate in moist chamber for 15 min. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 timesfor 2 minutes each	15 min
6. Reagents 2A, 2B: 2A: DAB Substrate(RTU) 2B: DAB Chromogen(20x)	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of **Reagent2B** (DAB Chromogen) to 1mL **Reagent 2A** (DAB Substrate). Mix well. Protect from light and use within 5 hours. b. Apply 2 drops or enough volume of DAB CHROMOGEN mixture to completely cover tissue. Incubate for 3-10 min. c. Rinse thoroughly with distilled water 4 times, 2 minutes each time. d. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 timesfor 2 minutes each.	3 - 10 min
7. Reagent 3A: DS-RR-Block A (RTU)	a. Apply 2 drops or enough volume of **Reagent 3A** (DS-RR-Block A) tocover the tissue completely. Mix well on the slide and Incubate in moist chamber for 30 min. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	30 min
8. Reagent 3B: DS-RR-Block B (RTU)	a. Apply 2 drops or enough volume of **Reagent 3B** (DS-RR-Block B) tocover the tissue completely. Mix well on the slide and Incubate in moist chamber for 5 min. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	5 min
9. 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 rabbit primary antibody 2 to cover the tissue completely. b. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 timesfor 2 minutes each.	30 - 60 min.
10. Reagent 4: Rabbit AP polymer(RTU)	a. Apply 1drop (50µL) of Reagent 4 (Rabbit AP polymer) to cover each section. b. Incubate in moist chamber for 30 min. c. Wash with only 1xTBS-T 3 times for 2 minutes each.	15 min.
11. Reagent 5A, 5B, 5C **Reagent 5A:**Permanent Red Substrate (RTU) **Reagent 5B:**Permanent Red Activator (5x) **Reagent 5C:** Permanent Red Chromogen (100x)	a. Add 200µL of **Reagent 5B** (Activator) into 1mL of **Reagent 5A** (Substrate) and mix well. Add 10µL of **Reagent 5C** (Chromogen) intothe mixture and mix well. [Note: For fewer slides, add 100µL of Reagent 5B (Activator) into 500µL of **Reagent 5A** (Substrate) and mixwell. Add 5µL of **Reagent 5C** (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. c. Rinse well with distilled water.	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 ½ - 1 min.) d. Rinse well in distilled water.	
13. Reagent 6 NeoMount Universal (RTU)	a. Apply 2 drops (100µl) or enough volume of **Reagent 6** NeoMount Universal 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 minutesor leave at room temperature until slides are thoroughly dried. Hardened NeoMount Universal forms an impervious polymer barrierto organic solvent. Do not use oil directly on the top of dried NeoMount Universal.	30 min. in 40- 50°C oven Or overnightat 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. 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