NeoStain Poly DS Kit - for Goat and Rabbit antibody on Human/Rodent tissue (DAB/Permanent Red)

NB-23-00109

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#cat : NB-23-00109-3	**Size: 120 ml**
#cat : NB-23-00109-2	**Size: 36 ml**
#cat : NB-23-00109-1	**Size: 12 ml**

Storage: 2-8ºC

Intended use:

NeoStain Poly DS kit is designed to use with user supplied goat and rabbit primary antibodies, to detect two distinct antigens on human and mouse tissue or cell samples.

This kit has been tested in paraffin tissue. However, this kit can be used on frozen specimen and freshly prepared monolayer cell smears.

Double staining is one of most commonly methods used in immunohistostaining for revealing two distinct antigens in a single tissue. NeoStain Poly DS kit from NeoBiotech Labs supplies two polymer enzyme conjugates:HRP Polymer anti-Goat IgG and AP Polymer anti-Rabbit IgG with two substrates/chromogens, DAB (Brown)and Permanent Red (Red).

Simplified steps offer a convenient protocol as the enzyme conjugates are applied to the specimen simultaneously.

If only the anti-goat antigen is present, HRP polymer will result with DAB (brown) chromogen will be present and if only the anti-rabbit antigen is present, AP polymer will react only with GBI-Permanent Red (red) chromogen. When both rabbit and goat antigen is present both DAB and GBI-Permanent Red chromogen will be present.

NeoStain Poly DS kit is a non-biotin system, avoiding blocking steps for endogenous biotin non-specific binding.

Kit components:

Component No.	Content	6mL Kit	36mL Kit	120mL Kit
Reagent 1	Goat 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	Simpo-Mount (RTU)	15mL	18ml x 2	1.2ml

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 deparffinized with xylene and rehydrated with a graded series of alcohols 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. **DO NOT** let specimen or tissue dry during protocol.
7. 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 results.

Reagent	Staining Procedure	Incubation Time (Min.)
1. Peroxidase Blocking Reagent Not provided	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (NeoPure Dual Enzyme Block NB-23-00193 was Recommended) for 10 minutes. b. Rinse the slide using distilled water at least twice.	10 – 20 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 for 2 min., 3 times	
3. Primary Antibody Mix**: one Goat and one Rabbit antibody** Supplied by user	Note: Investigator needs to optimize dilution prior to double staining. a. Apply 2drops (100µL) or enough volume of goat and rabbit primary antibodies mixture to cover the tissue completely. Incubate in moist chamber for 30-60min. Recommend 30min to shorten total protocol time. b. Wash with PBS/0.05% Tween20 for 2min, 3 times	30-60min
4.Mix **Reagent 1:** Goat HRP Polymer (RTU) with **Reagent 2** Rabbit AP Polymer (RTU)	**Note**: Only make enough mixture for the experiment performed. Mixture is not stable for long term storage. Make sufficient polymer mixture by adding **Reagent 1** Goat HRP Polymer and **Reagent 2** Rabbit AP Polymer at 1:1 ratio, mix well. a. Apply 2 drops (100µL) or enough volume of the mixture to cover each section. b. Incubate in moist chamber for 15min. c. Wash with PBS/ 0.05% Tween20 for 2min, 3 times.	15 min.
5. Reagent 3A and 3B **Reagent 3A:** DAB Substrate (RTU**)** **Reagent 3B:** DAB Chromogen (20x)	**Note**: Make enough DAB mix by adding 1 drop of **Reagent 3B** DAB Chromogen in 1mL of **Reagent 3A** DAB Substrate. Mix well. Store at 4°C protecting from light and use within 7 hours. a. Apply 1 to 2 drops (50-100µL) of DAB working solution to cover the tissue completely. b. Incubate for 5min. c. Rinse slides with distilled water 2min 3 times, or running tap water for 1min.	5 min
6. Reagent 4A, 4B, 4C **Reagent 4A:** Permanent Red Substrate (RTU)	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	10 min

Reagent 4B: Permanent Red Activator (5x) **Reagent 4C:** Permanent Red Chromogen (100x)	buffer) and mix well. Add 5µL of **Reagent 4C** (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	
7. Counterstain (Optional) Not provided	a. Counterstain with 2 drops (100µL) or enough volume of counterstain solution to completely cover tissue. Incubate for 10-15sec. b. Rinse thoroughly with tap water for 2-3min. c. Rinse well in distilled water.	10 – 15 sec
8. Reagent 5: Simpo-Mount (RTU)	Apply 2 drops (100µL) or enough volume of Reagent 5 Simpo-Mount to cover tissue when tissue is wet. Rotate the slides to allow Simpo-Mount spread evenly.	

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 coversliped as well. However the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.
   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.

Precautions:

Please wear gloves, eye protection and take other necessary precautions. If any of the reagent come in contact with skin wash area completely with plenty of water and soap. If irritation develops seek medical attention.

FOR RESEARCH USE ONLY

Work Sheet for NB-23-00109 Kit

We designed this work sheet to help you keep 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.

- Used for tester to check "√ "each step during the experiment
- Steps follow after de-paraffinization
- Refer to insert for details of each step

NB-23-00109 Protocol is suitable when both rabbit and rabbit primary antibodies need or do not need pre-treatment step

Protocol Step	**NB-23-00109 Protocol**	**Experiment 1 Date:**	**Experiment 2 Date:**	**Experiment 3 Date:**	**Experiment 4 Date:**
Step 1	Peroxidase& alkaline phosphatase Block User supplied				
Step 2	HIER if needed User supplied				
Step 3	Gt 1°Ab & Rb 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** Goat HRP Polymer (RTU)& Rabbit AP Polymer (RTU) require mixing 30min				
Step 5	**Reagent 3A & Reagent 3B** DAB requires mixing 5min				

Step 6	**Reagent 4A, 4B & 4C** Permanent Red Requires mixing! 10min				
Step 7	Counter stain 10-15sec Hematoxylin User supplied				
Step 8	**Reagent 5** Simpo-Mount (RTU)				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Testing result