PolyStain DS Kit - for Goat and Rabbit antibody on Human

Rodent tissue (DAB/Fast-Red)

NB-23-00112- 3(120 ml)

NB-23-00112- 2(36 ml)

NB-23-00112- 1(12 ml)

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NB-23-00112-1; NB-23-00112-2; NB-23-00112-3

Storage: 2-8ºC

INTENDED USE:

PolyStain 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. PolyStain 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 Fast 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 Fast Red (red) chromogen. When both rabbit and goat antigen is present both DAB and Fast Red will be present. PolyStain DS Kit is a nonbiotin 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	Fast Red Chromogen	6 tablets	18 tablets	60 tablets
Reagent 4B	Fast Red Substrate(RTU)	5ml x 6	5ml x 18	5ml x 60
Reagent 5	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 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. 1. Peroxidase and Alkaline Phosphatase 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 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 for 2min,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 2 drops (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.	30min.

	b. Incubate in moist chamber for 30min. c. Wash with PBS/ 0.05% Tween20 for 2min, 3 times	
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: Fast Red Chromogen It takes about **30 minutes** to dissolve the tablet in the substrate buffer. Allow enough time to prepare.	a. Dissolve 1 tablet of **Reagent 4A** (Fast Red Chromogen) in 5ml **Reagent 4B** (Fast Red Substrate), vortex until the tablet dissolved completely. Use within 1 hour. b. Apply 2 drops (100µL) or enough volume of Fast Red working solution to completely cover the tissue. Incubate for 10-20 min, observe appropriate color development c. Rinse well with distilled water**. (Fast Red is alcohol soluble; do not dehydrate.)**	10 min
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.	
8. Reagent 5: NeoMount Universal (RTU)	a. 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. **DO NOT** coverslip. 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. Hardened NeoMount Universal forms an impervious polymer barrier to organic solvent. Do not use oil directly on the top of dried NeoMount Universal. **To coverslip see protocol note 2.**	30min in 40- 50°C oven Or: Overnight at 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. NeoMount Universal is an aqueous-based mounting media for immunohistochemistry. It is used as the permanent mounting media for alcohol soluble chromogens such as Fast 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.

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

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Work Sheet for NB-23-00112 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

Protocol Step	NB-23-00112 Protocol	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase& alkaline phosphatase Block				
Step 2	HIER if needed				
Step 3	Gt 1°Ab & Rb 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** Goat AP Polymer (RTU)& Rabbit HRP Polymer (RTU) require mixing 30min				
Step 5	**Reagent 3A & Reagent 3B** DAB requires mixing 5min				
Step 6	**Reagent 4A &Reagent 4B** Fast Red Requires mixing! 10min				

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Step 7	Counter stain Hematoxylin User supplied				
Step 8	**Reagent 5** NeoMount Universal (RTU) Do not coverslip!				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Testing result: