PolyStain Double Staining Kit - for 2 Rabbit antibody on Human/Mouse tissue (DAB/Fast Red)

NB-23-00108-1 (12ml)
NB-23-00108-2 (36ml)
NB-23-00108-3 (120ml)

PolyStain DS Kit – for 2 Rabbit antibody on Human / Mouse tissue for Immunohistochemistry Staining

(PolyStain HRP & AP double staining kit to detect two rabbit primary antibodies on human/mouse tissue with DAB (purple) and Fast Red (Red))

Cat# NB-23-00108-1 size : 6+6= 12ml (for 60 slides)*

Cat# NB-23-00108-2 size : 18+18= 36ml (for 180 slides)*

Cat# NB-23-00108-3 size : 60+60= 120ml (for 600slides)*

** if use 100µl per slide*

Introduction:

PolyStain HRP & AP double staining kit to detect two rabbit primary antibodies on human/mouse tissue with DAB (purple) and Fast Red (Red)

Intended Use:

The PolyStain DS Kit - RR-Hu/Ms D 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 on frozen 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 [1, 2]. PolyStain DS Kit - RR-Hu/Ms D supplies two polymer enzyme conjugates: HRP polymer anti-Rabbit IgG and AP polymer anti-Rabbit IgG with two distinct substrates/chromogens, DAB (brown color, use with HRP polymer anti-Rabbit IgG) and Fast Red (red color, use with AP polymer anti-Rabbit IgG). PolyStain DS Kit - RR-Hu/Ms D is non-biotin system that avoids endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	12ml Kit	36ml Kit	120ml Kit
Reagent 1	HRP polymer anti-Rabbit IgG (RTU)	6ml	18ml	60ml
Reagent 2A	DAB substrate buffer (RTU)	12ml	18ml	60ml
Reagent 2B	DAB chromogen (20X)	1ml	2ml	60ml
Reagent 3A	DS-RR Blocker A	6ml	18ml	60ml
Reagent 3B	DS-RR Blocker B	6ml	18ml	60ml
Reagent 4	AP polymer anti-Rabbit IgG (RTU)	6ml	18ml	60ml
Reagent 5A	Fast Red chromogen tablets	6 tablets	18 tablets	60 tablets
Reagent 5B	Fast Red substrate buffer	5ml x 6	5ml x 18	5ml x 60
Reagent 6	NeoBio Mount Universal (RTU)	12ml	18ml	60ml

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 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. It takes about 30 minutes to dissolve Fast Red tablet into the substrate buffer. Make sure to start preparing Fast Red solution near the end of the secondary antibody incubation.
7. Proceed IHC staining: DO NOT let specimen or tissue dry from this point on.
8. 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.

Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. Neo Biotech sells 10xTBS-T for your convenience (NB-23-00201)

Reagent	**Staining Procedure**	**Incubation Time (Min.)**
1. Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided. We recommend using **NeoPure Dual Enzyme Block NB-23-00193-1 or -2**. Fast, easy and it will block endogenous alkaline phosphatase	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent. We recommend **NeoPure Dual Enzyme Block NB-23-00193-1 or -2**. b. Rinse the slide using 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-T containing 0.05% Tween-20 or 1X TBS-T (See note 8 above); 3 times for 2 minutes each.	
3. Preblock (optional)	a. For paraffin section, Improved formula saves the need for a preblock step. (optional) b. For frozen tissue, preblock may or may not be required depending on fixative. (Neoblock catalogue No.:NB-23-00169-3 was recommended)	
4. Rabbit Antibody 1 (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 1 to cover the 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: HRP polymer anti-Rabbit IgG (RTU)	a. Apply 2 drops or enough volume of Reagent 1 HRP polymer anti-Rabbit IgG to cover each section. b. Incubate in moist chamber for 20-30 min. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	20-30 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 Reagent 2B to 1 ml Reagent 2A. 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 times for 2 minutes each..	3-10 min

7. Reagent 3A: DS-RR Blocker A	a. Apply 2 drops or enough volume of Reagent 3A DS-RR Blocker A to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 30 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 min.,3 times.	30 min
8. Reagent 3B: DS-RR Blocker B	a. Apply 2 drops or enough volume of DS-RR Blocker to cover 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 times for 2 minutes each.	30-60 min
10. Reagent 4: AP polymer anti-Rabbit IgG (RTU)	a. Apply 2 drops or enough volume of Reagent 4 AP Polymer anti-Rabbit IgG to cover each section. b. Incubate in moist chamber for 20-30 min. c. Wash with 1X TBS-T only; 3 times for 2 minutes each.	20-30 min
11. Reagent 5A, 5B: Fast Red Chromogen: It takes about 30 minutes to dissolve the tablet in the substrate buffer. Allow enough time to prepare.	a. Dissolve 1 Reagent 5A Fast Red tablet in 5ml Reagent 5B Fast Red substrate buffer, vortex until the tablet dissolved completely. Use within 1 hour. b. Apply 2 drops (100 µl) or enough volume of Fast Red work 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.)	10-20 min
12. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100 µl) 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: NeoBio Mount Universal solution (RTU)	a. Apply 2 drops (100 µl) or enough volume of Reagent 6 to cover tissue when tissue is wet. Rotate the slides to allow NeoBio Mount 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 NeoBio Mount Universal forms an impervious polymer barrier to organic solvent. Do not use oil directly on the top of dried NeoBio Mount Universal.	30 min. 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 effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.

2. NeoBio Mount Universal is water-based mounting medium for immunohistology. It may be used as the permanent mounting media. It does not need coverslip. However, if you need to coverslip, after NeoBio Mount Universal dried, dip the slide in xylene and take out immediately. Apply NeoBio Mount Perm (Catalog No. NB-23-00156) on the tissue and place cover glass on the slide. Store it after dry completely.

Precautious:

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

Remarks:

For research use only.

Storage:

Store at 4°C.

References:

1. De Pasquale A, Paterlini P, Quaglino D.Immunochemical demonstration of different antigens in single cells in paraffin-embedded histological sections. Clin Lab Haematol. 1982;4(3):267-72.
2. Polak J. M and Van Noorden S. Introduction to Immunocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997