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

(DAB/Permanent Red)

NB-23-00090- 3(120 ml)

NB-23-00090- 2(36 ml)

NB-23-00090- 1(12 ml)

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NB-23-00090-1; NB-23-00090-2; NB-23-00090-3

Storage: 4-8ºC

INTENDED USE:

The PolyStain DS Kit is designed to use with user supplied mouse and rabbit antibody to detect two distinct antigens on human tissue or cell samples.

This kit has been tested in paraffin tissue, but may also be used on frozen specimens and freshly prepared monolayer cell smears. Double staining is one of most common methods used in immunohistostaining for revealing two distinct antigens in a single tissue. The PolyStain DS Kit from Golden Bridge International supplies two polymer enzyme conjugates: AP-Polymer anti-Mouse IgG and HRP Polymer anti-Rabbit IgG with two distinct chromogens, DAB (brown) and Permanent Red (red).

User may apply the two enzyme conjugates onto the specimen at the same time and mix them on the slide. Simplified steps allow for a much faster protocol than sequential procedures.

The PolyStain DS Kit is a 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-Mouse IgG (RTU)	6mL	18mL	60 mL
Reagent 2	AP-Polymer anti-Rabbit IgG (RTU)	6mL	18mL	60mL
Reagent 3A	DAB Substrate (RTU)	12mL	18mLx2	120 mL
Reagent 3B	DAB Chromogen (20x)	1.5mL	2mL	6mL
Reagent 4A	Permanent Red Substrate (RTU)	15mL	18mLx2	120mL
Reagent 4B	Permanent Red Activator (5x)	3mL	7.2mL	12mLx2
Reagent 4C	Permanent Red Chromogen (100x)	150µL	360µL	1.2mL
Reagent 5	NeoMount Universal (RTU)	12 mL	18 mL x 2	120 mL

HRP = Horseradish Peroxidase AP = Alkaline Phosphatase Ms = Mouse Rb = Rabbit

RECOMMENDED PROTOCOL:

1. Proper 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 deparaffinize 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. 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. This will generate false positive and/or false negative signal.
7. The fixation, tissue section 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.
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.

Reagent	Staining Procedure	Incubation Time (Min.)
1.Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided 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**. b. Rinse the slide using distilled water.	10 min.
2. Antigen retrieval if needed: Refer to primary antibody data sheet.	a. Refer to primary antibody data sheet for antigen retrieval methods 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)	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.	

4. Primary Antibody Mix**: Mix one Mouse and one Rabbit primary antibody** Supplied by user.	**Notes**: Investigator needs to optimize dilution prior to double staining. a. Apply 2 drops or enough volume of both Primary Antibody 1and Antibody 2 to cover the tissue completely. Mix well on the slide and 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 and 2: **Reagent 1:** HRP Polymer anti-Mouse IgG (RTU) **Reagent 2:** AP Polymer antiRabbit IgG (RTU)	**Note**: Make sufficient polymer mixture by adding **Reagent 1** HRP Polymer anti-Rabbit IgG and **Reagent 2** AP Polymer anti-Mouse IgG at 1:1 ratio, mix well. Do Not Mix More than you need for the experiment because the polymer mixture may not be as stable as non-mixed polymer. a. Apply 1 to 2 drops (50-100µL) of the mixture to cover the tissue completely. b. Incubate in moist chamber for 30 min. c. Wash with PBS-T containing 0.05% Tween-20 or 1X TBS-T; 3 times for 2 minutes each.	30 min
6. Reagents 3A, 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. Use within 7 hours. a. Apply 1 to 2 drops (50-100µL) of your DAB mixture to cover the tissue completely. b. Incubate for 5min. c. Rinse slides in multiple changes of distilled water 3 times, 2 each time or under running tap water for 1 minute. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	5 min
7. Reagent 4A, 4B,4C: **Reagent 4A:** Permanent Red Substrate (RTU) **Reagent 4B:** Permanent Red Activator (5x**)** **Reagent 4C:** Permanent Red Chromogen (100x) **(To get maximum sensitivity of AP polymer, Please repeat chromogen step)**	Note: Shake Permanent Red Activator before adding into Permanent Red Substrate. 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.] 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. To increase AP signal aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the Permanent Red working solution to completely cover the tissue for additional 5 to 10min. c. Rinse well with distilled water.	**8** min

8. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 5 seconds. DO NOT over stain with hematoxylin b. Rinse thoroughly with tap water for 1 minute c. Put slides in PBS for 5 seconds to blue, **DO NOT** over blue. d. Rinse well in distilled or tap water for 1 minute	
9. Reagent 5: NeoMount Universal (RTU)	a. Apply 2 drops (100µl) or enough volume of **Reagent 5** to cover tissue when tissue is wet. Rotate the slides to allow NeoMount Universal spread evenly. 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.	

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. 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

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