PolyStain Double Staining Kit - for Mouse and Rabbit Antibody on Mouse tissue

NB-23-00094-1 (12ml)
NB-23-00094-2 (36ml)
NB-23-00094-3 (120ml)

PolyStain DS Kit – Mouse and Rabbit antibody on Mouse tissue for Immunohistochemistry Staining

Polymer-HRP & AP double staining kit to detect a rabbit and a mouse primary antibody on mouse tissue with DAB (Brown) and Permanent Red (Red)

NB-23-00094-1 *size : 6+6 = 12ml* *(for* 120 slides*)*
NB-23-00094-2 *size : 18+18 = 36ml* *(for 360* slides*)*
NB-23-00094-3 *size : 60+60 = 120ml* *(for* 1200 slides*)*
** if use 100µL per slide*

Intended Use:

The PolyStain DS Kit - MR-Ms A is designed to use with user supplied mouse and rabbit primary antibody to detect two distinct antigens on mouse tissue or cell samples. The kit can be used on frozen specimens, paraffin–embedded tissues, or freshly prepared monolayer cell smears. The kit is designed not to give background on most mouse strains however there may be some mouse strains especially when using frozen that require additional blocking; we recommend PureStain Mouse-on-Mouse Kit (NB-23-00076-x) to improve specificity of the mouse primary antibody on mouse tissue.
Double staining is one of most common methods used in immunohistostaining that allows detection of two distinct antigens in a single tissue[1, 2]. PolyStain DS Kit - MR-Ms A supplies two polymer enzyme conjugates: Mouse HRP Polymer and Rabbit AP Polymer with two distinct substrates/chromogens, DAB (brown color, use with the Mouse HRP Polymer) and Permanent Red (red color, use with the Rabbit AP Polymer). A Primer step is used to increase specificity of antibody staining. Both enzyme conjugates are applied to the specimen at the same time and mixed on the slide. This kit offers simplified steps that make for a quicker and easier protocol than that used in a sequential procedure. The PolyStain DS Kit is non-biotin system that avoids endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Mouse Primer (RTU)	12mL	18mLx2	120mL
Reagent 2	Mouse HRP Polymer (RTU)	6mL	18mL	60mL
Reagent 3	Rabbit AP Polymer (RTU)	6mL	18mL	60mL
Reagent 4A	DAB Substrate (RTU)	15mL	18mLx2	120mL
Reagent 4B	DAB Chromogen (20x)	1.5mL	2mL	6mL
Reagent 5A	Permanent Red Substrate (RTU)	15mL	18mLx2	120mL
Reagent 5B	Permanent Red Activator (5x)	3mL	7.2mL	12mLx2
Reagent 5C	Permanent Red Chromogen (100x)	150µL	360µL	1.2mL
Reagent 6	NeoBio Mount Universal (RTU)	12mL	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 deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.

4. Cell smear samples should be made up to as much of a monolayer as possible to obtain s atisfactory 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 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. (10xTBS-T wash buffer, 500ml, NB-23-00201)

Reagent	**Staining Procedure**	**Incubation Time**
1. Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided We recommend using **NeoPure Dual Enzyme Block** (NB-23-00193-1) Fast, easy and it will block endogenous alkaline phosphatase	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent. b. We recommend **NeoPure Dual Enzyme Block (NB-23-00193-1, 18ml / NB-23-00193-2 100ml)**. Rinse the slide using distilled water at least twice.	10 min.
2. HIER Pretreatment: Refer to Ab 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 7 above)**; 3 times for 2 minutes each.	
3. **PureStain Mouse-on-Mouse Blocking solution A (NB-23-00076-1)** Not provided	a. Add 2 drops (100µL) or enough volume of **PureStain Mouse-on-Mouse Blocking solution A** to cover the tissue section and Incubate.	30 min.
(optional see protocol note 2)	b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	
4. **PureStain Mouse-on-Mouse Blocking solution B** Not provided **(optional see protocol note 2)**	a. Add 2 drops (100µL) or enough volume of **PureStain Mouse-on-Mouse Blocking solution B** to cover the tissue section and Incubate. **Do not** exceed 5min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	5 min.
5. Mouse antibody 1 and Rabbit antibody 2: Supplied by user	Note: InvInvestigator needs to optimize dilution and incubation times prior to double staining a. Apply 2 drops or enough volume of both Ms Primary Antibody 1 and Rb Primary 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.
6. **Reagent 1** Mouse Primer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 1** (Mouse Primer) to cover the tissue section and Incubate Room Temperature for 10-15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	15min
7. **Reagent 2 and 3** **Reagent 2 :** Mouse HRP Polymer(RTU) **Reagent 3:** Rabbit AP Polymer(RTU)	**Note:** Make sufficient polymer mixture by adding **Reagent 2** (Mouse HRP Polymer) and **Reagent 3** (Rabbit AP Polymer) at 1:1 ratio, mix well. Do Not mix more than you need for the experiment because the polymer mixture is not stable for long term storage. 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.

8. **Reagent 4A and 4B** **Reagent 4A:** DAB Substrate (RTU) **Reagent 4B:** DAB Chromogen (20x)	a. Add 1 drop of **Reagent 4B to** 1 mL of **Reagent 4A**. Mix well. Protect from light and use within 7 hours at 4 ºC. b. Apply 2 drops or enough volume of DAB Chromogen working solution to completely cover tissue. Incubate for 3-10 min. c. Rinse thoroughly with distilled water. d. Wash with 1xTBS-T only, 3 times for 2 minutes each. **DAB is a predicted carcinogen, wear gloves.**	5 min.
9. **Reagent 5A, 5B, 5C** **Reagent 5A:** Permanent Red Substrate (RTU) **Reagent 5B:** Permanent Red Activator (5x) **Reagent 5C:** 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 5B** (Activator) into 1mL of **Reagent 5A** (Substrate buffer) and mix well. Add 10µL of **Reagent 5C** (Chromogen) into the mixture and mix well. [Note: For fewer slides, Add 100µL of **Reagent 5B** (Activator) into 500µL of **Reagent 5A** (Substrate buffer) and mix well. Add 5µL of **Reagent 5C** (Chromogen) into the mixture and mix well.] b. Apply 2 drops (100µL) or enough volume of GBI-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 GBI-Permanent Red working solution to completely cover the tissue for additional 5 to 10min.** c. Rinse well with distilled water.	10 min. + 5-10 min
10. 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 30 - 60 sec.) d. Rinse well in distilled water.	
11. **Reagent 6:** NeoBio Mount Universal (RTU) **To coverslip see protocol note 3**.	a. Apply 2 drops (100µL) or enough volume of **Reagent 6** (NeoBio Mount Universal) to cover tissue when tissue is wet. Rotate the slides to allow NeoBio Mount 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. PureStain Mouse-on-Mouse Kit block the anti-mouse secondary has been absorbed to rat serum resulting in most mouse strains having no background, however some mouse strains may need additional blocking. PureStain Mouse-on-Mouse Kit (NB-23-00076-1) works very well on frozen tissue.
3. 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 (NeoBio Mount Perm Cat# 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!

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

Related products

Product	Catalog No.	Size
10xTBS-T wash buffer	NB-23-00201	500ml
NeoPure Dual Enzyme Block	NB-23-00193-1 / -2	18ml / 100ml
PureStain Mouse-on-Mouse Blocking	NB-23-00076-1 / -2 / -3	110ml / 18ml / 6 ml
NeoBio Mount Perm (Organic)	NB-23-00156	18ml
NeoBio Mount Universal (Aqueous)	NB-23-00157-1 / -2	100ml / 18ml