PureStain Mouse-on-Mouse Kit, AP Detection System with Permanent Red

NB-23-00073

PureStain Mouse-on-Mouse Kit, AP Detection System with Permanent Red

Improved formula with Permanent Red for more sensitive detection of mouse primary antibodies on mouse tissue, biotin free

#Cat: NB-23-00073-2 **Size: 18ml*, with chromogen** (sufficient for 180 slides**)
#Cat: NB-23-00073-3 **Size: 6ml*, with chromogen** (sufficient for 60 slides**)

*Total volume of polymer Conjugates
** If use 100µLper slide

Intended Use:

Antigen detection of primary antibodies from the same host species as the test tissue can generate high background when indirect IHC detection method are used. This severely limits the use of mouse monoclonal antibodies on mouse tissues. Neo Biotech PureStain Mouse-on-Mouse Kit – AP Detection System with Permanent Red is designed for staining mouse antibodies on mouse tissues. The new formula allows better detection of mouse primary antibodies without increasing the background. The PureStain Mouse-on-Mouse Kit – AP Detection System with Permanent Red uses a special blocking buffer, polymeric AP-linked secondary antibody as well as mouse antibody enhancer to to increase sensitivity to detect mouse primary antibodies without increasing background. This technology provides excellent sensitivity and specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotins.

Kit Components:

Component No.	Content	6mL Kit	18mL Kit
Reagent 1	MS Blocking A (RTU)	6mL	18mL
Reagent 2	MS Blocking B (RTU)	6mL	18mL
Reagent 3	Mouse Antibody Enhancer(RTU)	6mL	18mL
Reagent 4	Polymer AP for Mouse(RTU)	6mL	18mL
Reagent 5A	Permanent Red Substrate (RTU)	7mL	18mL
Reagent 5B	Permanent Red Activator (5x)	1.4mL	3.6mL
Reagent 5C	Permanent Red Chromogen (100x)	70µL	180µL

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 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 (slide treated with Isotype control reagent), and negative control.
6. Start staining procedures: 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 inhibitor the activity of the alkaline phosphatase

   Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. (We recommend 10xTBS-T NB-23-00201)

Neo Biotech
74 rue des Suisses – 92000 Nanterre

Reagent	Staining Procedures	Incubation Time
1. Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (NeoPure Dual Enzyme Block # NB-23-00193-1 / -2 was Recommended) for 10 minutes. b. Rinse the slide using distilled water at least twice.	10min
2. HIER Pretreatment: refer to antibody supplier's data	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to primary antibody datasheet. b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** (See note 7 above) 3 times for 2 minutes each.	
3. **Reagent 1:** Ms Blocking A (RTU)	a. Add 2 drops or enough volume of **Reagent 1** MS Blocking A to cover the tissue section completely and Incubate 30 min. b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** 3 times for 2 minutes each.	30min.
4. **Reagent 2:** Ms Blocking B (RTU)	a. Add 2 drops or enough volume of **Reagent 2** MS Blocking B to cover the tissue section completely and Incubate 5 min. b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** 3 times for 2 minutes each.	5min
5. Primary antibody: Supplied by user.	**Note: With the PureStain Mouse-on-Mouse Kit, the concentration of primary antibody has to be optimized by user.** a. Apply 2 drops or enough volume of Primary antibody to cover the tissue section completely. Incubate in moist chamber for 30-60 min. b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** 3 times for 2 minutes each.	30-60min.
6. **Reagent 3:** Mouse Antibody Enhancer (RTU)	a. Add 2 drops or enough volume of **Reagent 3** Mouse Antibody Enhancer to cover the tissue section completely and Incubate for 10 min, longer incubation may increase background. b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** 3 times for 2 minutes each.	15min
7. **Reagent 4:** Polymer AP for Mouse (RTU)	a. Apply 2 drops or enough volume of **Reagent 4** Polymer AP for Mouse to cover the tissue section completely and incubate 10 minutes. b. Wash with **1xTBS-T** 3 times for 2 minutes each.	15min.
8. **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, 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 2B** (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 Permanent Red working solution to completely cover the tissue for additional **10min**. c. Rinse well with distilled water.	10 min +10min

9. Hematoxylin: **Supplied by user**	a. Counterstain with 2 drops or enough volume to cover tissue completely and wait about 10-20 econds. b. Rinse thoroughly under tap water for 1-2 min. c. Put slides in PBS until show blue color (about 30-60 seconds) d. Rinse well in distilled water	
10. Mounting media: **Supplied by user**	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. NeoBio Mount AQ: Cat.# NB-00155-3 (18ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. NeoBio Mount Universal: Cat.# NB-23-00157-2 (18ml), or NB-23-00157-1 (100ml), universal permanent mounting medium. Can be used with or without cover slip	

Protocol Notes:

1. The fixation, tissue slide thickness, 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. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.
5. 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:
Please wear gloves and take other necessary precautions.

Remarks: For research use only.

Storage: Store at 4°C.

References:

1. Bisgaard K, Pluzed KP. Use of polymer conjugates in immunohitochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates. Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungry, October 20-25, 1996.
2. Shi ZR. Itzkowitz SH, Kim YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcimoma tissues. J Hitochem Cytochem 36:317-322,

Related products

Product	Catalog No.	Size
PureStain Mouse-on-Mouse HRP for DAB Bulk Kit	NB-23-00074-1	110mL (w/o chromogen)
PureStain Mouse-on-Mouse HRP for DAB	NB-23-00074-5 /-4	6mL / 18mL
PureStain Mouse-on-Mouse Blocking A & B	NB-23-00076-1 /-2	100mL / 18mL
PolyStain 2-Step Plus Kit, HRP, RAT-NM with DAB for Rat antibody on Mouse Tissue	NB-23-00052-3 /-2	6mL / 18mL
PolyStain 2-Step Plus Kit, AP, RAT-NM with P.Red for Rat antibody on Mouse Tissue	NB-23-00070-2 /-3	6mL / 18mL
PolyStain 2-Step Plus Kit, HRP Mouse-NR, with DAB for Mouse antibody on Rat tissue	NB-23-00053-3 /-2	6mL / 18mL
PolyStain 2-Step Plus Kit, AP Mouse-NR with P.Red for Mouse antibody on Rat tissue	NB-23-00071-2 /-3	6mL / 18mL