PureStain Mouse-on-Mouse Kit, HRP for AEC

NB-23-00075-1

PureStain Mouse-on-Mouse Kit, HRP for AEC, no chromogen

#Cat: NB-23-00075-1 Size: 110 ml no chromogen

Storage: 4- 8ºC

Intended Use:

Antigen detection of primary antibodies from the same host species as the test tissue can generate high background when indirect IHC detection methods are used for the screen. This severely limits the use of mouse monoclonal antibodies on mouse tissues.

Neo Biotech Labs Inc's PureStain Mouse HRP-Polymer Detection System 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 HRP Polymer DAB Kit uses a special blocking buffer, antibody enhancer and polymeric HRP linked secondary antibody 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:

Catalog Number	Content	NB-23-00075 1
Reagent 1	MS Blocking A(RTU)	110mL
Reagent 2	MS Blocking B(RTU)	110mL
Reagent 3	Mouse Antibody Enhancer(RTU)	110mL
Reagent 4	Polymer HRP anti-Mouse (RTU)	110mL
Reagent 5A	AEC Substrate (20x)	Not Included
Reagent 5B	AEC Chromogen (20x)	Not Included
Reagent 5C	Hydrogen Peroxide (20x)	Not Included

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 deparaffinized 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. PureStain Mouse is a time sensitive protocol; please adhere to protocol incubation times to prevent background from occurring. Increasing incubation times of reagents 3 and 4will increase background in the plasma of some mouse strains.

Reagent	Staining Procedures	Incubation Time (Min.)
1. Peroxidase blocking reagent: Supplied by user.	a. Apply 2 drops (100µL) or enough volume of Peroxidase blocking reagent (Ready-to-use 3% H2O2 solution) to cover the tissue section and incubate b. Rinse the slide using distilled water.	10 min.
2. HIER Pretreatment: refer to antibody supplier's data	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; 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-T containing 0.05% Tween-20; 3 times for 2 minutes each	30 min.
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-T containing 0.05% Tween-20; 3 times for 2 minutes each.	5 min.

5. Primary antibody: Supplied by user.	**Note**: **With the PureStain 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-T containing 0.05% Tween-20;3 times for 2 minutes each	30-60 min
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 15 minutes, longer incubation may increase background. b. Wash with PBS-T containing 0.05% Tween-20; 3 times for 2 minutes each	15 min
7. Reagent 4: Polymer HRP anti-Mouse (RTU)	a. Apply 2 drops or enough volume of Reagent 4 Polymer HRP Antibody to cover the tissue section completely and incubate 15 minutes, longer incubation may increase background. b. Wash with PBS-T containing 0.05% Tween-20; 3 times for 2 minutes each	15 min
8. Reagents 5A, 5B, 5C **5A:** AEC Substrate (20x) **5B:** AEC Chromogen (20x) **5C:** Hydrogen Peroxide (20x)	a. Prepare 1mL of distilled water. Adding 1 drop AEC Substrate (Reagent 5A) in 1mL of distilled water. Mix well. b. Add 1 drop or 2 drops (for higher contrast and sensitivity) of AEC Chromogen (**Reagent 5B)** and 1 drop of Concentrated Hydrogen Peroxide (**Reagent 5C**) to the pre-diluted Reagent 5A. Mix well. c. Add 2 drops (100uL) of the fresh made AEC mixture on the slides and incubate in an enclosed chamber at room temperature about 5-10 minutes and observe color development. d. When appropriate color is developed, rinse under tap water gently for about 1-2 minutes. e. Keep away from light during operation and use the prepared AEC solution within 1 hour	10 min.
9. Hematoxylin: Supplied by user	a. Counterstain with 2 drops or enough volume to cover tissue completely and wait about 10-20 seconds. b. Rinse thoroughly under tap water for 1-2 minutes. 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: NeoMount AQ: Cat. No. **NB-23-00155-3** (18mL) NeoMount Universal: Cat. No. **NB-23-00157-2** (18mL)	

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. 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

Related Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
PureStain Mouse-on-Mouse Kit, AP with Fast Red	NB-23-00073-5 NB-23-00073-4	6 mL 18 mL		NeoStain Poly 2-Step Plus Kit, HRP, Rat-NM,	NB-23-00064-3 NB-23-00064-2	6 mL 18 mL
				NeoStain Poly 2-Step Plus Kit, AP, Rat-NM, with Permanent Red	NB-23-00070-2 NB-23-00070-3	6 mL 18 mL
PureStain Mouse-on-Mouse Kit, AP with Permanent Red	NB-23-00073-3 NB-23-00073-2	6 mL 18 mL		NeoStain Poly 2-Step Plus Kit, HRP, Mouse-NR, with DAB	NB-23-00053-3 NB-23-00053-2	6 mL 18 mL
PureStain Mouse-on-Mouse Kit Blocking A & B solutions	NB-23-00076-1 NB-23-00076-2	110 mL 18 mL		NeoStain Poly 2-Step Plus Kit, HRP, Mouse-NR, with AEC	NB-23-00065-3 NB-23-00065-2	6 mL 18 mL
NeoStain Poly 2-Step Plus Kit, HRP, Rat-NM, with DAB	NB-23-00052-3 NB-23-00052-2	6 mL 18 mL		NeoStain Poly 2-Step Plus Kit, AP, Mouse-NR, with Permanent	NB-23-00071-2 NB-23-00071-3	6 mL 18 mL

Precautions:

Handle all specimens as potential infectious materials, wear gloves, eye protection, and proper protection for clothes when handling all reagents.

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