PureStain Mouse-on-Mouse HRP-Polymer DAB Detection Kit

NB-23-00074

PureStain Mouse-on-Mouse HRP-Polymer DAB Detection Kit

(Improved formula with new protocol for more sensitive detection of mouse antibodies on mouse tissue, biotin free)

#Cat: NB-23-00074-1 Size : 110ml, no chromogen
#Cat: NB-23-00074-2 Size : 60ml, no chromogen
#Cat: NB-23-00074-3 Size : 60ml, with DAB
#Cat: NB-23-00074-4 Size : 18ml, with DAB
#Cat: NB-23-00074-5 Size : 6ml, with DAB

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. PureStain Mouse-on-Mouse HRP-Polymer Detection Kit is designed for staining mouse antibodies on mouse tissues. The new formula allows better detection of mouse primary antibodies without increasing the background. PureStain Mouse-on-Mouse HRP-Polymer Detection 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:

Component No.	Content	NB-23-00074-5	NB-23-00074-4	NB-23-00074-3	NB-23-00074-2	NB-23-00074-1
Reagent 1	MS Blocking A (RTU)	6mL	18mL	60mL	60mL	110mL
Reagent 2	MS Blocking B (RTU)	6mL	18mL	60mL	60mL	110mL
Reagent 3	Mouse Antibody Enhancer (RTU)	6mL	18mL	60mL	60mL	110mL
Reagent 4	Polymer HRP anti-Mouse (RTU)	6mL	18mL	60mL	60mL	110mL
Reagent 5A	DAB Substrate (RTU)	12mL	2x15mL	60mL	Not Included	Not Included
Reagent 5B	DAB Chromogen (20x)	1.5mL	2mL	3mL	Not Included	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 4 will increase background in the plasma of some mouse strains.

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Reagent	Staining Procedures	Incubation Time
1. Peroxidase blocking reagent: Supplied by user.	a. Apply 2 drops (100µL) or enough volume of Peroxidase blocking reagent (Ready-to-use 3% H_2O_2 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** **5A:** DAB Substrate (RTU) **5B:** DAB Chromogen(20x)	a. Add 1 drop (or 2 drops for higher contrast) of **Reagent 5B** DAB Chromogen in 1mL of **Reagent 5A** DAB Substrate. Mix well and store at 4°C. Protect from light and use within 4 hours. b. Apply 2 drops (100µL) or enough volume of DAB working solution to completely cover tissue and incubate 5 minutes. c. Wash with distilled water for 2 min, 3 times.	5 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: NeoBio Mount AQ: Cat. No. NB-23-00155-3 (18mL) NeoBio Mount 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

Precautions:

You should handle all kit components as potentially hazardous materials please wear gloves, eye protection, appropriate lab entire in addition to lab coat when handling any or all reagents.

Storage:

Store at 4°C.

Remarks:

For research use or investigation only. Not for diagnostic or therapeutic use.

Related Products:

PureStain Human-on-Human Kit, HRP with DAB	NB-23-00082-2 / -3	6ml / 18ml
PureStain Mouse-on-Mouse Kit, HRP with AEC	NB-23-00075-3 / -2	6ml / 18ml
PureStain Mouse-on-Mouse Kit, AP with Permanent Red	NB-23-00073-3 / -2	6ml / 18ml
PureStain Mouse-on-Mouse Kit, Blocking A & B solutions	NB-23-00076-1 / -2	110ml / 18ml
NeoStain Poly 2-Step Plus Kit, HRP, with DAB, for Rat antibody on Mouse Tissue	NB-23-00052-3 / -2	6ml / 18ml
NeoStain Poly 2-Step Plus Kit, HRP, with AEC, for Rat antibody on Mouse Tissue	NB-23-00064-3 / -2	6ml / 18ml
NeoStain Poly 2-Step Plus Kit, AP, with Permanent Red, for Rat antibody on Mouse Tissue	NB-23-00070-3 / -2	6ml / 18ml
NeoStain Poly 2-Step Plus Kit, HRP, with DAB, for Mouse antibody on Rat Tissue	NB-23-00053-3 / -2	6ml / 18ml
NeoStain Poly 2-Step Plus Kit, HRP, with AEC, for Mouse antibody on Rat Tissue	NB-23-00065-3 / -2	6ml / 18ml
NeoStain Poly 2-Step Plus Kit, AP, with Permanent Red, for Mouse antibody on Rat Tissue	NB-23-00071-3 / -2	6ml / 18ml

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