Klear Mouse HRP-Polymer DAB Detection System

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

Storage: 4-8ºC

Catalog No.:	D52-6	6mL ☐
	D52-18	18mL ☐
	D52-60D	60mL ☐
	D52-110	110mL ☐

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. GBI Labs Inc's Klear 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 Klear Mouse HRP-Polymer Detection kit uses a special blocking buffer, polymeric HRP-linked secondary antibody, antibody enhancer, and now a polymer enhancer to increase sensitivity to the 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	60mL Kit	110mL Kit
Reagent 1	MS Blocking A(RTU)	6mL	18mL	60mL	110mL
Reagent 2	MS Blocking B(RTU)	6mL	18mL	60mL	110mL
Reagent 3	Mouse Antibody Enhancer(RTU)	6mL	18mL	60mL	110mL
Reagent 4	Polymer HRP Antibody (RTU)	6mL	18mL	60mL	110mL
Reagent 5	Polymer Enhancer	6mL	18mL	60mL	110mL
Reagent 6A	DAB Substrate(RTU)	12mL	2x15mL	60mL	Not Included
Reagent 6B	DAB Chromogen(20x)	1.5mL	2mL	3mL	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. Klear Mouse is a time sensitive protocol; please adhere to protocol incubation times to prevent background from occurring. Increasing incubation times of reagents 3, 4, and 5 will increase background in the plasma of some mouse strains
8. **Note:** This protocol requires you use TBS-T as the wash buffer to get high sensitivity and clean background.
   1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6

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 TBS-T 2 min., 3 times.	
3. **Reagent 1:** MS Blocking A	a. Add 2 drops or enough volume of **Reagent 1** MS Blocking A to cover the tissue section completely and Incubate 30 min. b. Rinse with TBS-T for 2 min., 3 times.	30 min.
4. **Reagent 2:** MS Blocking B	Add 2 drops or enough volume of **Reagent 2** MS Blocking B to cover the tissue section completely and Incubate 5 min. b. Wash in TBS-T for 2 min., 3 times.	5 min
5. Primary antibody: Supplied by user.	**Note: With the Klear 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.	30-60 min.

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	b. Wash with TBS-T for 2 min., 3 times.	
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 minutes, longer incubation may increase background. b. Wash with TBS-T for 2 min., 3 times.	10 min
7. **Reagent 4:** Polymer HRP Antibody (RTU) **Do Not Wash** Go directly to step 8	a. Apply 2 drops or enough volume of **Reagent 4** Polymer HRP Antibody to cover the tissue section completely and incubate 10 minutes, longer incubation may increase background. **Do not wash after this step just remove excess liquid.** **Tap off Polymer HRP to remove liquid and add Reagent 5**	10 min.
8. **Reagent 5** Polymer Enhancer (RTU)	a. Apply 2 drops or enough volume of **Reagent 5** Polymer Enhancer to cover the tissue section completely and incubate 10 minutes, longer incubation may increase background. b. Wash with TBS-T for 2 min., 3 times.	10min
9. **Reagents 6A, 6B** **6A:**DAB Substrate (RTU) **6B:**DAB Chromogen(20x)	a. Add 1 drop (or 2 drops for higher contrast) of **Reagent 6B** DAB Chromogen in 1mL of **Reagent 6A** DAB Substrate. Mix well. 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.
10. 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	
11. Mounting media: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: GB-Mount: Cat. No. E01-18 (18mL) Simpo-Mount: Cat.No. E03-18 (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
Klear Mouse AP Fast Red kit	D50-6 / D50-18	6mL / 18mL
Klear Mouse HRP with AEC Kit	D53-6 / D53-18	6mL / 18mL
Klear Mouse AP AP-Red Kit	D51-6 / D51-18	6mL / 18mL
Klear Mouse Blocking A & B	D54-110 / D54-18	110mL / 18mL
Polink-2 Plus HRP RAT-NM DAB kit for Rat antibody on Mouse Tissue	D46-6 / D46-18	6mL / 18mL
Polink-2 Plus HRP RAT-NM AEC kit for Rat antibody on Mouse Tissue	D48-6 / D48-18	6mL / 18mL
Polink-2 Plus AP RAT-NM kit for Rat antibody on Mouse Tissue	D67-18 / D67-6	6mL / 18mL
Polink-2 Plus HRP Mouse-NR DAB kit for Mouse antibody on Rat tissue	D58-6 / D58-18	6mL / 18mL
Polink-2 Plus HRP Mouse-NR AEC kit for Mouse antibody on Rat tissue	D59-6 / D59-18	6mL / 18mL
Polink-2 Plus AP Mouse-NR kit for Mouse antibody on Rat tissue	D65-18 / D65-6	6mL / 18mL

Precautious:

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

Remarks: For research use only.

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