GBI Labs
GOLDEN BRIDGE INTERNATIONAL

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Klear Mouse HRP AEC Detection System

(For mouse primary antibody on mouse tissue, biotin free)

Storage: 4-8ºC

Catalog No.:			
	D53-6	6 ml	☐
	D53-18	18ml	☐
	D53-110	110ml	☐

Intended Use:

Antigen detection with primary antibody of the same species as the test tissue yields high background when indirect detection method is used. This severely limits the use of murine monoclonal antibody on mouse tissues. Golden Bridge International's Klear Mouse HRP-Polymer Detection System is designed for staining mouse antibodies on mouse tissues. The Klear Mouse HRP-Polymer Detection kit uses a special blocking buffer, polymeric HRP-linked secondary antibody as well as mouse antibody enhancer to detect mouse primary antibody that bound to the mouse tissue. This technology provides excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotins. Klear Mouse HRP-Polymer AEC Detection System is super sensitive to AEC chromogen. **With Klear mouse system, the primary antibody will need to be screened at higher concentration(2-10 fold) than regular mouse detection kits.**

Kit Components:

Cat. No.	Reagent 1 MS Blocking A (RTU)	Reagent 2 MS Blocking B (RTU)	Reagent 3 MS Antibody Enhancer (RTU)	Reagent 4 Polymer-HRP Conjugate (RTU)	Reagent 5A AEC Buffer (20x)	Reagent 5B AEC chromogen (20x)	Reagent 5C H_2O_2 (20x)
D53-6	6ml	6 ml	6 ml	6 ml	1ml	2ml	1 ml
D53-18	18ml	18ml	18ml	18ml	2 ml	4ml	2ml
D53-110	110ml	110ml	110ml	110ml	Not included	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.

Reagent	Staining Procedures	Incubation Time (Min.)
1. Peroxidase blocking reagent: Supplied by user.	a. Apply 2 drops (100 µl) 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 2 min., 3 times.	
3. **Reagent 1:** MS Blocking A (RTU)	a. Add 2 drops or enough volume of **Reagent 1** MS Blocking A Solution to cover the tissue section completely and Incubate 30 min. b. Rinse with PBS for 2 min., 3 times.	30 min.
4. **Reagent 2:** Ms Blocking B (RTU)	Add 2 drops or enough volume of **Reagent 2** MS Blocking B Solution to cover the tissue section completely and Incubate 5 min. b. Rinse with PBS for 2 min., 3 times.	5 min
5. Primary antibody: Supplied by user.	**Note: With Klear mouse system, the primary antibody will need to be screened at higher concentration (2-10 fold) than regular mouse detection kits.** 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. Rinse with PBS for 2 min., 3 times.	30-60 min.
6. **Reagent 3:** Mouse Antibody Enhancer	Add 2 drops or enough volume of **Reagent 3** Mouse Antibody Enhancer to cover the tissue section completely and Incubate for 10 min	10 min

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(RTU)	b. Rinse with PBS for 2 min., 3 times.	
7. **Reagent 4:** Polymer-HRP Conjugate (RTU)	a. Apply 2 drops or enough volume of **Reagent 4** Polymer-HRP Conjugate to cover the tissue section completely and incubate 10 minutes. b. Rinse with PBS for 2 min., 3 times.	10 min.
8. **Reagents 5A, 5B, 5C:** 5A: AEC Buffer (20x) 5B: AEC Chromogen (20x) 5C: H_2O_2 (20x)	a. Prepare 1 ml of distilled water. Adding 1 drop AEC buffer (**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	5 -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 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: 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 HRP for DAB Bulk Kit	D52-110	110ml (chromogen is not included)
Klear Mouse HRP with DAB kit	D52-6 / D52-18	6ml / 18ml
Klear Mouse AP Fast-Red Kit	D50-6 / D50-18	6ml / 18ml
Klear Mouse AP AP-Red Kit	D51-6 / D51-18	6ml / 18ml
Klear Mouse Blocking A & B	D54-100 / D54-18	100ml / 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 and protection cloth when handling all reagents.

Remarks:

For research use only.

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