

Klear Mouse AP-Polymer GBI-Permanent Red Detection System

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

Storage: 4-8°C

Catalog No.: D51-6 6mL* 60slides**
 D51-18 18mL* 180slides**
 D51-60D 60mL* 600slides**
 D51-110 110mL* 1100slides**
**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. GBI Labs inc Klear Mouse AP 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 AP-Polymer Detection kit uses a special blocking buffer, polymeric AP-linked secondary antibody as well as mouse antibody enhancer 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	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 AP for Mouse(RTU)	6mL	18mL	60mL	110mL
Reagent 5A	GBI-Permanent Red Substrate (RTU)	7mL	18mL	60mL	NA
Reagent 5B	GBI-Permanent Red Activator (5x)	1.4mL	3.6mL	12mL	NA
Reagent 5C	GBI-Permanent Red Chromogen (100x)	70µL	180µL	0.6mL	NA

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. 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.**

Reagent	Staining Procedures	Incubation Time
1. Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (Klear Dual Enzyme Block E36 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 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. b. Wash with PBS/ 0.05% Tween20 or 1xTBS-T 3 times for 2 minutes each.	30-60min.
6. Reagent 3:	a. Add 2 drops or enough volume of Reagent 3 Mouse Antibody Enhancer to cover the tissue	15min

Mouse Antibody Enhancer (RTU)	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.	
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: GBI-Permanent Red Substrate (RTU) Reagent 5B: GBI-Permanent Red Activator (5x) Reagent 5C: GBI-Permanent Red Chromogen (100x) To get maximum sensitivity of AP polymer, Repeat chromogen step	Note: Shake GBI-Permanent Red Activator before adding into GBI-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 GBI-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 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: 1. GB-Mount: Cat. No. E01-18 (18mL), for AEC, AP-Red, GBI-Permanent Red and AP-blue. 2. Simpo-Mount: Cat.No. E03-18 (18mL), or E03-100 (100ml), universal permanent mounting medium	

Protocol Notes:

- 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.
- 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.
- Do not mix reagents from different lot.
- Do not allow the slides to dry at any time during staining.
- GBI-Permanent Red is insoluble in organic solvent and can be coverslipped 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.

- 1x 80% Ethanol 20 seconds;
- 1x 95% Ethanol 20 seconds;
- 3x 100% Ethanol 20 seconds each;
- 1x 100% Xylene 20 seconds;
- Add 1 drop of xylene based mountant (Cat. No. O-Mount, E02-18) and coverslip. Press to push the air bubble out.

CAUTION: DO NOT dehydrate in xylene longer than 20 seconds! It will erase GBI-Permanent Red stain!

Related Products:

Product	Catalog No.	Size
Klear Mouse HRP for DAB Bulk Kit	D52-110	110mL (w/o chromogen)
Klear Mouse HRP with DAB kit	D52-6 / D52-18	6mL / 18mL
Klear Mouse HRP with AEC Kit	D53-6 / D53-18	6mL / 18mL
Klear Mouse AP Fast Red Kit	D50-6 / D50-18	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

Precautions:

Please wear gloves, eye protection and take other necessary precautions. If any of the reagent come in contact with skin wash area completely with plenty of water and soap. If irritation develops seek medical attention.

Remarks:

For research use only.

References:

- De Pasquale A, Paterlini P, Quaglini D. *Immunochemical demonstration of different antigens in single cells in paraffin-embedded histological sections.* Clin Lab Haematol. 1982;4(3):267-72.
- Polak J. M and Van Noorden S. *Introduction to Immunocytochemistry Second Edition.* Bios Scientific Publishers. P41-54. 1997