GBI Labs
GOLDEN BRIDGE INTERNATIONAL

Klear Human HRP-Polymer DAB Detection System

(For Detection of Human Primary Antibodies on Human Tissues, Biotin Free)

Storage: 4-8ºC

Catalog No.:	D103-6	6 ml	☒
	D103-18	18ml	☐
	D103-110	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 screening human antibody on human tissues. GBI Labs Klear Human HRP-Polymer Detection System is designed for staining human primary antibodies on human tissues without background staining. The Klear Human HRP-Polymer Detection kit provides special blocking buffers, polymeric HRP-linked secondary antibody as well as human primer in a ready to use system. This technology requires an overnight pre-incubation with primary antibody that results in 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.

Kit Components:

Component No.	Content	6ml Kit	18ml Kit	110ml Kit
Reagent 1	Human Primer (RTU)	6ml	18ml	110ml
Reagent 2	Quenching Buffer (5xConcentrate)	1.5ml	2.3mlx2	13mlx2
Reagent 3	Hu Blocking A (RTU)	6ml	18ml	110ml
Reagent 4	Hu Blocking B (RTU)	6ml	18ml	110ml
Reagent 5	Human HRP Polymer (RTU)	6ml	18ml	110ml
Reagent 6A	DAB Substrate (RTU)	12ml	15x2ml	Not Included
Reagent 6B	DAB Chromogen (20xConcentrate)	1.5ml	2ml	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 into a monolayer as much 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 Day 1 Primary Human Antibody Preparation**	**Incubation Time**
Dilute primary antibody in **Reagent 1** Human Primer (RTU)	**Reagent 1** (Human Primer) is at ready to use concentration. Dilute primary antibody in Human primer at user determined primary antibody concentration. Mix gently for 30sec to 1min. Recommend only diluting amount needed for experiment. Place at 4C overnight.	O/N at 4℃
Reagent	**Staining Procedures Day 2**	**Incubation Time**
Prepare slides	See Recommended Protocols above	
1. Peroxidase blocking reagent: Supplied by user.	a. Apply 2 drops 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 move to pretreatment step.	10 min.

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	No Pretreatment then do step c. c. Wash with PBS/0.05% tween20 for 2 min., 3 times.	
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/0.05% tween20 for 2 min., 3 times.	
3. Bring to Room temp (Hu primary Ab diluted in Reagent 1) add **Reagent 2 (Quenching Buffer 5x Concentration)**	Remove **Hu primary Ab diluted in Reagent 1** from fridge and allow mix to come to room temperature. a. After **Hu primary Ab diluted in Reagent 1** has come to room temperature add **Reagent 2** into mixture. b. Take the total volume of (**Hu primary Ab diluted in Reagent 1**) _____µl ÷ 5 **=** _____ µl amount of **Reagent 2 (Quenching Buffer 5x Concentration). Incubate at room temperature for 15-30 min.** c**.** Store at 4C or on ice until you reach step 6.	15-30 min
4. **Reagent 3:** Hu Blocking A (RTU)	a. Add 2 drops or enough volume of **Reagent 3** (Hu Blocking A) to cover the tissue section completely and Incubate 30 min. b. Wash with PBS/0.05% tween20 for 2 min., 3 times.	30 min.
5. **Reagent 4:** Hu Blocking B (RTU)	a. Add 2 drops or enough volume of **Reagent 4** (Hu Blocking B) to cover the tissue section completely and Incubate 5 min. b. Wash with PBS/0.05% tween20 for 2 min., 3 times.	5 min
6. Add Primary Ab mixture from step 3	**Note:** Optimized incubation time should be tested. We find that incubating 2-4 hours at room temperature or overnight at 4C works great without background. a. Add 2 drops or enough volume of mixture from **step 3 {(Primary Ab) / (Reagent 1 Human Primer) /(Reagent 2 Quenching Buffer)}** to cover the tissue section completely and Incubate 30-60 min. (Recommend 2 hours, but it will increase background) b. Wash with PBS/0.05% tween20 for 2 min., 3 times.	30-60 min
7. **Reagent 5:** Human HRP Polymer (RTU)	a. Apply 2 drops or enough volume of **Reagent 5** (Human HRP Polymer) to cover the tissue section completely and incubate 10 minutes. b. Wash with PBS/0.5% tween20 for 2 min., 3 times.	10 min.
8. **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 7 hours. b. Apply 2 drops (100 µl) or enough volume of pre-mixed DAB to completely cover tissue and Incubate 5 minute. 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. Wash 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) 2. Simpo-Mount: Cat.No. E03-18 (18ml)	

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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	D54-100 / D54-18	100ml / 18ml
Klear Rat HRP DAB kit	D98-6 / D98-18	6ml / 18ml
Klear Rat HRP AEC kit	D99-6 / D99-18	6ml / 18ml
Klear Rat AP Fast-Red	D100-6 / D100-18	6ml / 18ml
Klear Rat AP AP-red	D101-6 / D101-18	6ml / 18ml
Klear Rat Blocking A & B	D102-18	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:

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.

Remarks:

For research use only.

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