Klear Human AP-Polymer Detection System
(with GBI Permanent Red)

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

Storage: 4-8ºC

Catalog No.:		
	D106-6	6mL ☐
	D106-18	18mL ☐
	D106-110	110mL ☐
	D106-110D	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 AP-Polymer Detection System is designed for generating staining with the alkaline phosphatase (AP) enzyme of human primary antibodies on human tissues without background staining. The Klear Human AP-Polymer Detection kit provides special blocking buffers, polymeric AP-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. **Note:** This kit is recommended for cytoplasmic and membrane bound antigens other patterns of staining have not tested.

Kit Components:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit	110mL-D Kit
Reagent 1	Human Primer (RTU)	6mL	18mL	110 mL	110 mL
Reagent 2	Quenching Buffer (5x)	1.5mL	2.3mLx2	13 mL x2	13 mL x2
Reagent 3	Hu Blocking A (RTU)	6mL	18mL	110 mL	110 mL
Reagent 4	Hu Blocking B (RTU)	6mL	18mL	110 mL	110 mL
Reagent 5	Human AP Polymer (RTU)	6mL	18mL	110 mL	110 mL
Reagent 6A	GBI-Permanent Red Substrate (RTU)	7mL	18mL	Not Included	110 mL
Reagent 6B	GBI-Permanent Red Activator (5x)	1.4mL	2 x 1.8mL	Not Included	12 mL x2
Reagent 6C	GBI-Permanent Red Chromogen (100x)	70µL	180µL	Not included	1.2 mL

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.
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
8. Serum blocking before primary antibody incubation for GBI's Polink-1, Polink-2, and Polink-2Plus is not required because all our antibody conjugates are absorbed to human serum.

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 your human primary antibody in the 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 4C

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Reagent	Staining Procedures Day 2	Incubation Time
Prepare slides	See Recommended Protocols above	
1. Phosphatase blocking reagent: Supplied by user. We recommend using GBI Labs **Klear Dual Block**- **E36-xx** which blocks both endogenous phosphatase and peroxidase enzymes.	a. Apply 2 drops or enough volume of phosphatase blocking reagent (GBI Labs Klear Dual Block- **E36-xx**) to cover the tissue section and incubate b. Rinse the slide using distilled water move to pretreatment step. *No Pretreatment then do step c.* c. Wash **1X TBS-T** for 2 minutes, 3 times. (See note 7 for **TBS-T** ingredients in recommended protocol above.)	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 **1X TBS-T** for 2 minutes, 3 times. (See note 7 for **TBS-T** ingredients in recommended protocol above.)	
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 on ice until you reach step 6. Note: Do not to quench for longer than 1 hour.	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 **1X TBS-T** for 2 minutes, 3 times. (See note 7 for **TBS-T** ingredients in recommended protocol above.)	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 **1X TBS-T** for 2 minutes, 3 times. (See note 7 for **TBS-T** ingredients in recommended protocol above.)	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 **1X TBS-T** for 2 minutes, 3 times. (See note 7 for **TBS-T** ingredients in recommended protocol above.)	30-60 min
7. **Reagent 5:** Human AP Polymer (RTU)	a. Apply 2 drops or enough volume of **Reagent 5** (Human AP Polymer) to cover the tissue section completely and incubate 10 minutes. b. Wash **1X TBS-T** for 2 minutes, 3 times. (See note 7 for **TBS-T** ingredients in recommended protocol above.)	10 min.

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8. **Reagent 6A, 6B, 6C** **Reagent 6A:** GBI-Permanent Red Substrate (RTU) **Reagent 6B:** GBI-Permanent Red Activator (5x) **Reagent 6C:** GBI-Permanent Red Chromogen (100x) **To get maximum sensitivity of AP polymer, Repeat the chromogen step**	**Note:** Shake GBI-Permanent Red Activator before adding into GBI-Permanent Red Substrate. a. Add 200µL of **Reagent 6B** (Activator) into 1mL of **Reagent 6A** (Substrate buffer) and mix well. Add 10µL of **Reagent 6C** (Chromogen) into the mixture and mix well. [Note: For fewer slides, Add 100µL of **Reagent 6B** (Activator) into 500µL of **Reagent 6A** (Substrate buffer) and mix well. Add 5µL of **Reagent 6C** (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 signa,** aspirate or tap off chromogen and apply 2-3 drops (100µL) of the GBI-Permanent Red working solution again to completely cover the tissue for additional **5 to 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. Wash thoroughly under tap water for 1-2 min. c. Put slides in TBS not tween until show blue color (about 30-60 seconds) d. Rinse well in distilled water	
10. Mounting Medium User supply	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)	

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	6mL / 18mL
Klear Human HRP DAB kit	D103-6/D103-18/D103-110	6mL/18mL/110mL
Klear Human HRP AEC kit	D104-6/D104-18/D104-110	6mL/18mL/110mL
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, and appropriate lab entire in addition to lab coat when handling any or all reagents.

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Remarks: For research use only.

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