Polink DS-MRt-Hu A Kit for Immunohistochemistry Staining

Polymer HRP and AP Double Staining Kit to Detect a Mouse & a Rat Primary Antibodies on Human Tissue with DAB (Brown) and GBI-Permanent Red (Red)

Storage: 2-8ºC

Catalog No.:
- ☐DS209A-6 12mL* for 120 slides**
- ☐DS209A-18 36mL* for 360 slides**
- ☐DS209A-60 120mL* for 1200 slides**

**Total volume of polymer Conjugates*
*** if using 100µL per slide*

Intended Use:
The **Polink DS-MRt-Hu A Kit** is designed for use with user supplied mouse and rat primary antibodies to detect two distinct antigens on human tissue or cell samples. This kit has been tested on paraffin embedded tissue. However, this kit can be used to stain frozen specimen and/or freshly prepared monolayer cell smears.

Double staining is a common method used in immunohistostaining that allows detection of two distinct antigens in a single tissue[1, 2]. **Polink DS- MRt-Hu A Kit** from GBI labs supplies the user with two polymer enzyme conjugates: anti-Mouse IgG (minimal cross reaction to rat) HRP polymer and anti-rat IgG(minimal cross reaction to mouse) AP polymer with two distinct substrates/chromogens, GBI-Permanent Red and DAB. GBI-Permanent Red reacts with anti-Rat AP polymer conjugate to produce the red color. DAB chromogen reacts with anti-Mouse HRP polymer conjugate to produce the brown color. A Primer step is used to increase specificity of antibody staining. **Polink DS-MRt-Hu A Kit** is a non-biotin system that avoids the extra steps involved in blocking non-specific binding due to endogenous biotin.

Kit Components:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Rat Primer (RTU)	12mL	18mLx2	120mL
Reagent 2	Rat AP Polymer (RTU)	6mL	18mL	60mL
Reagent 3	Mouse HRP Polymer (RTU)	6mL	18mL	60mL
Reagent 4A	DAB Substrate (RTU)	15mL	18mLx2	120mL
Reagent 4B	DAB Chromogen (20x)	1.5mL	2mL	6mL
Reagent 5A	GBI-Permanent Red Substrate (RTU)	15mL	18mLx2	120mL
Reagent 5B	GBI-Permanent Red Activator (5x)	3mL	7.2mL	12mLx2
Reagent 5C	GBI-Permanent Red Chromogen (100x)	150µL	360µL	1.2mL
Reagent 6	Simpo Mount (RTU)	15mL	18mLx2	120mL

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 falling off.
3. Paraffin embedded sections must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made up to as much of a monolayer as possible to obtain satisfactory results.
5. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
6. Proceed IHC staining: 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 inhibit the activity of the alkaline phosphatase. **Note: 1X TBS-T** =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. GBI sells 10xTBS-T for your convenience (B11xx)

Step/Reagent	Staining Procedure	Incubation Time
1. Peroxidase and Alkaline Phosphatase Blocking Reagent Not provided We recommend using **GBI Dual Block E36xx.** Fast, easy and it	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent. We recommend **GBI Dual Block E36xx**. b. Rinse the slide using distilled water at least twice.	10min

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

will block endogenous alkaline phosphatase		
2. HIER Pretreatment: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T(See note 7 above)**; 3 times for 2 minutes each.	
3. Primary Antibody Mix: **one Mouse and one Rat primary antibody** Supplied by user	***Notes:*** Investigator needs to optimize primary antibody titer and incubation time prior to double staining. a. Apply 2 drops or enough volume of Mouse and Rat primary antibody mixture to cover the tissue completely. Incubate in moist chamber for 30-60 min. Recommend 30min to shorten total protocol time. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30-60min
4. **Reagents 1:** Rat Primer (RTU)	a. Apply 2 drops or enough volume of **Reagent 1** Rat Primer to cover the tissue completely. Incubate in moist chamber for 10min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	10min
5. **Reagents 2 & 3:** **Reagents 2 :** Rat AP Polymer(RTU) **Reagents 3:** Mouse HRP Polymer(RTU)	**Note:** Make sufficient polymer mixture by adding **Reagent 2** (Rat AP Polymer) and **Reagent 3** (Mouse HRP Polymer) at 1:1 ratio, mix well. a. Apply 1 to 2 drops (50-100µL) of the mixture to cover each section. b. Incubate in moist chamber for 30 min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each. **Make enough mixture for the experiment. Do not make extra volume as mixture is not stable for long term storage .**	30min
6. **Reagent 4A and 4B** **Reagent 4A:** DAB Substrate(RTU) **Reagent 4B:** DAB Chromogen (20x)	**Note:** Make enough DAB mix by adding 1 drop of Reagent **4B** (DAB Chromogen) in 1mL of **Reagent 4A** (DAB Substrate). Mix well. Use within 7 hours store at 4C. a. Apply 1 to 2 drops (50-100µL) of your DAB mixture to cover the tissue completely. b. Incubate for 5min. c. Rinse thoroughly with distilled water. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	5min
7. **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, Please repeat chromogen step)**	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 5B** (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 5 to 10min.** c. Rinse well with distilled water.	10min
8. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds. b. Rinse thoroughly with tap water for 2-3 min. c. Put slides in PBS until show blue color (about 30 - 60 sec.) d. Rinse well in distilled water.	
9. **Reagent 6:** Simpo Mount (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 6** Simpo-Mount to cover tissue when tissue is wet. Rotate the slides to allow Simpo-Mount spread evenly.	

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

Protocol Notes:

1. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting the result.
2. **GBI-Permanent Red** is insoluble in organic solvent and can be coversliped as well. however the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.
   a. 1x 80% Ethanol 20 seconds;
   b. 1x 95% Ethanol 20 seconds;
   c. 3x 100% Ethanol 20 seconds each;
   d. 1x 100% Xylene 20 seconds;
   e. Add 1 drop of xylene based mountant (Cat. No. O-Mount, E02-18) and coverslip. Press to push the air bubble out.

Precautious:

DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

Remarks:

For research use only.

References:

1. De Pasquale A, Paterlini P, Quaglino D.*Immunochemical demonstration of different antigens in single cells in paraffin-embedded histological sections.* Clin Lab Haematol. 1982;4(3):267-72.

2. Polak J. M and Van Noorden S. Introduction to Immnocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

Work Sheet for DS209A Kit

We designed these work sheets to help you track of each step. When staining fails these sheets help our technical support staff to pinpoint the problem.
To insure that all steps are done properly, we recommend that the user fill in the actual time of their experimental step and any variation. Results will vary if time recommendations are not followed. RTU translates to ready to use.

- Used for tester to check "√ "each step during the experiment
- Steps follow after de-paraffinization
- Refer to insert for details of each step

DS209A Protocol is suitable when both mouse and rat primary antibodies need or do not need pre-treatment step.

Protocol Step	DS209A Protocol Reagent / Time	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase or Alkaline Phosphatase Block User supplied				
Step 2 (Optional)	HIER if needed User supplied (up to 60 min)				
Step 3	Mouse 1°Ab & Rat 1°Ab mix (30-60 min.)				
Step 4	**Reagent 1** Rat Primer RTU (10min)				
Step 5	**Reagent 2**&**Reagent 3** Rat AP Polymer & Mouse HRP Polymer require mixing (30min) Rinse with distilled water.				
Step 6	**Reagent 4A & 4B** DAB Requires mixing! (5 min.)				
Step 7	**Reagent 5A, 5B& 5C** GBI-Permanent Red Requires mixing! (10 min)				
Step 8	Counter stain User supplied				
Step 9	**Reagent 6** Simpo-Mount (RTU)				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Result:

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com