Polink DS-MRt-Hu B Kit for Immunohistochemistry Staining

Polymer HRP and AP Double Staining Kit to Detect a Mouse & a Rat Primary Antibodies on Human Tissue with BCIP/NBT (Purple) and AEC (Red)

Storage: 2-8ºC

Catalog No.: ☐DS209B-6 12ml* for 120 slides**
☐DS209B-18 36ml* for 360 slides**
☐DS209B-60 120ml* for 1200 slides**
**Total volume of polymer Conjugates*
*** if using 100µl per slide*

Intended Use:
The **Polink DS-MRt-Hu B Kit** is designed for use with user supplied mouse and rat primary antibodies to detect two distinct antigens on human tissue or cell samples. The advantage of the A kit series is that it will allow you to visualize when two proteins are co localized by producing a third color blue purple. Specimens can be frozen or paraffin embedded, 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 B 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, BCIP/NBT and AEC. BCIP-NBT reacts with anti-Rat AP polymer conjugate to produce the purple color. AEC chromogen reacts with anti-Mouse HRP polymer conjugate to produce the Red color. **Polink DS-MRt-Hu B 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	Mouse HRP Polymer (RTU)	6ml	18ml	60ml
Reagent 2	Rat AP Polymer (RTU)	6ml	18ml	60ml
Reagent 3	BCIP-NBT(RTU)	15ml	18mlx2	120ml
Reagent 4A	AEC Substrate (20x)	1ml	2ml	6ml
Reagent 4B	AEC Chromogen (20x)	2ml	4ml	12ml
Reagent 4C	Hydrogen Peroxide (20x)	1ml	2ml	6ml
Reagent 5	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 will block endogenous alkaline phosphatase	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.	10-20min
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.	

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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 & 2:** **1:** Mouse HRP Polymer(RTU) **2:** Rat AP Polymer(RTU)	**Note:** Make sufficient polymer mixture by adding **Reagent 1** (Mouse HRP Polymer) and **Reagent 2** (Rat AP 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 **1X TBS-T only**; 3 times for 2 minutes each.. d. Rinse with distilled water. **Make enough mixture for the experiment. Do not make extra volume as mixture is not stable for long term storage .**	30min
5. **Reagent 3** BCIP/NBT Chromogen (RTU)	a. Apply 2 drops or enough volume of **Reagent 3** (BCIP/NBT Chromogen) to completely cover tissue. Incubate for 3-10 min. b. Rinse thoroughly with distilled water. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	3-10 min
6. **Reagent 4A, 4B, 4C:** **4A:**AEC Substrate (20x) **4B:**AEC Chromogen (20x) **4C:**Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of **Reagent 4A** to 1ml distill water. Mix well . Add 2 drops of **Reagent 4B** and 1 drop of **Reagent 4C** to diluted AEC Substrate. Mix well. Keep away from light and use within 1 hour. b. Apply 2 drops (100µl) or enough volume of AEC working solution to completely cover the tissue. Incubate for 5-15 min, observe appropriate color development. c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate.**)	5-15 min
7. 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.	
8. **Reagent 5:** Simpo-Mount (RTU) **To coverslip see protocol note 2**.	a. Apply 2 drops (100µl) or enough volume of **Reagent 5** (Simpo-Mount) to cover tissue when tissue is wet. Rotate the slides to allow Simpo-Mount spread evenly. DO NOT coverslip. b. Place slides horizontally in an oven at 40-50°C for at least 30 minutes or leave it at room temperature until slides are thoroughly dried. Hardened Simpo-Mount forms an impervious polymer barrier to organic solvent. Do not use oil directly on the top of dried Simpo-Mount.	30 min. in 40-50°C oven Or: overnight at room temperature

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. Simpo-Mount is a water-based mounting medium for immunohistochemistry. It is used as the permanent mounting media for alcohol soluble chromogens such as AP-Red, AEC, and BCIP. Simpo-Mount does not use a coverslip. However, if you need to coverslip your tissue, after Simpo-Mount has dried, dip the slide in xylene (1 to 2 seconds), apply an organic mounting solution (such as O-Mount, Cat# E02-18), and place cover glass on the slide. Store slides after they have dried completely.

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

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Work Sheet for DS209B 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

Protocol Step	DS209B 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**& **Reagent 2** Mouse HRP Polymer & Rat AP Polymer require mixing (30min)				
Step 5	**Reagent 3** BCIP/NBT (RTU) (3-10 min)				
Step 6	**Reagent 4A, 4B, & 4C** AEC Requires mixing! (5-15 min)				
Step 7	Counter stain (Do not over counter stain) Hematoxylin User supplied				
Step 8	**Reagent 5** Simpo Mount (RTU) Do not coverslip!				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

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