Polink DS-MRt-Ms B Kit

(Polymer-HRP & AP double staining kit)
(Detects rat and mouse primary antibodies on mouse tissue with BCIP/NBT (Purple) and AEC (Red))

Storage: 2-8ºC

Catalog No.
- ☐ DS210B-6 6mL* 60 slides**
- ☐ DS210B-18 18mL* 180 slides**
- ☐ DS210B-60 60mL* 600slides**

**Total volume of polymer Conjugates*
***If use 100µL per slide*

Intended Use:
The **Polink DS-MRt-Ms B Kit** is designed to use with user supplied mouse and rat primary antibody to detect two distinct antigens on mouse tissue or cell samples. DS210 kits can be used on frozen specimens, paraffin–embedded tissues, or freshly prepared monolayer cell smears. DS210 kits is designed not to give background on most mouse strains.
Double staining is one of most common methods used in immunohistostaining that allows detection of two distinct antigens in a single tissue[1, 2]. **Polink DS-MRt-Ms B Kit** from GBI Labs-Inc supplies two polymer enzyme conjugates: Mouse HRP (AEC) Polymer and Rat AP Polymer with two distinct substrates/chromogens, AEC (red color, use with the Mouse HRP Polymer) and BCIP/NBT Red (purple color, use with the Rat AP Polymer). A Primer step is used to increase specificity of antibody staining. This kit offers simplified steps that make for a quicker and easier protocol than that used in a sequential procedure. **Polink DS-MRt-Ms B Kit** is non-biotin system that avoids endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	DS210B-6	DS210B-18	DS210B-60
Reagent 1	Rat AP Polymer (RTU)	6mL	18mL	60mL
Reagent 2	BCIP/NBT (RTU)	7mL	18mL	60mL
Reagent 3A	DS-MRt Blocker A (RTU)	6mL	18mL	60mL
Reagent 3B	DS-MRt Blocker B (RTU)	6mL	18mL	60mL
Reagent 4	Mouse Primer (RTU)	6mL	18mL	60mL
Reagent 5	Mouse HRP AEC Polymer (RTU)	6mL	18mL	60mL
Reagent 6A	AEC Substrate (20x)	1mL	1mL	3mL
Reagent 6B	AEC Chromogen (20x)	2mL	2mL	6mL
Reagent 6C	Hydrogen Peroxide (20x)	1mL	1mL	3mL
Reagent 7	Simpo-Mount (RTU)	6mL	18mL	60mL

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 with 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 (B11).

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. b. Rinse the slide with distilled water at least twice.	10 min.
2. HIER Pretreatment: Refer to Antibody data sheet	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to antibody datasheet 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. Rat primary antibody: Supplied by user	**Note:** Investigator needs to optimize the primary antibodies dilution and incubation time prior to double staining. a. Apply 2 drops or enough volume of rat primary antibody to cover the tissue completely. Mix well on the slide and incubate in moist chamber for 30-60 min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30-60 min.

9620 Medical Center Drive, Suite 200, Rockville, MD 20850, USA Phone: 1-301-340-3188 Fax: 301-340-9254

4. **Reagent 1:** Rat AP Polymer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 1** (Rat AP Polymer) to cover the tissue section and Incubate Room Temperature for 10- 15minutes. b. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	15 min
5. **Reagents 2:** BCIP/NBT Chromogen (RTU)	a. Apply 2 drops or enough volume of **Reagents 2** (BCIP/NBT Chromogen) to completely cover tissue. Incubate for 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.	10 min
6. **Reagent 3A:** DS-MRt Blocker A (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 3A** DS-MRt Block A to cover the tissue section and incubate. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30 min.
7. **Reagent 3B:** DS-MRt Blocker B (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 3B** DS-MRt Block B to cover the tissue section and incubate. **Do not** exceed 5min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	5 min.
8. Mouse primary antibody: Supplied by user	**Note:** Investigator needs to optimize the primary antibodies dilution and incubation time prior to double staining. a. Apply 2 drops or enough volume of mouse primary antibody to cover the tissue completely. Mix well on the slide and incubate in moist chamber for 30-60 min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30-60 min.
9. **Reagent 4:** Mouse Primer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 4** (Mouse Primer) to cover the tissue section and Incubate Room Temperature for 15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	15 min
10. **Reagent 5:** Mouse HRP AEC Polymer (RTU)	a. Add 2 drops (100µL) or enough volume of **Reagent 5** (Mouse HRP(AEC) Polymer) to cover the tissue section and incubate at Room Temperature for 15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30 min.
11. **Reagents 6A, 6B, 6C:** **Reagent 6A:** AEC Substrate (20x) **Reagent 6B:** AEC Chromogen (20x) **Reagent 6C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of **Reagent 6A** and 1 drop or 2 drops (for higher sensitivity and contrast) of **Reagent 6B** and 1 drop of **Reagent 6C** to 1ml distill water. Mix well. Keep away from light and use within 1 hour. b. Apply 2 drops (100µl) or enough volume of pre-mixed AEC solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate**)	10 min
12. 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 - 60sec) d. Rinse well in distilled water.	
13. **Reagent 7:** Simpo-Mount (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 7** (Simpo-Mount) to cover tissue when tissue is wet. Rotate the slides to allow Simpo- Mount spread evenly. 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. **To coverslip see protocol note 3 below.**	

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 water-based mounting medium for immunohistology. It may be used as the permanent mounting media. It does not need coverslip. However, if you need to coverslip, after Simpo-Mount dried, dip the slide in xylene and take out immediately. Apply O-Mount (organic mounting solution, Catalog No. E02-15) on the tissue and place cover glass on the slide. Store it after dry completely.

Precautions:

Please wear gloves and take other necessary precautions.

Remarks:

For research use only.

9620 Medical Center Drive, Suite 200, Rockville, MD 20850, USA Phone: 1-301-340-3188 Fax: 301-340-9254

Work Sheet for DS210B 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 ensure 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 de-paraffinization
- Refer to insert for details of each step

Step/ Protocol	Protocol DS210B	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase or Alkaline Phosphatase Block User supplied recommend E36				
Step 2	HIER if needed Refer to datasheet				
Step 3	Rat 1°Ab (30-60 min.)				
Step 4	**Reagent 1** Rat AP Polymer (15 min) (Wash with TBS-T only)				
Step 5	**Reagent 2** BCIP/NBT (10min)				
Step 6	**Reagent 3A** DS-MRt Block A (RTU) 30min				
Step 7	**Reagent 3B** DS-MRt Block B (RTU) 5min				
Step 8	Mouse 1°Ab (30-60 min.)				
Step 9	**Reagent 4** Mouse Primer RTU (15 min)				
Step 10	**Reagent 5** Mouse HRP(AEC) Polymer (15 min)				
Step 11	**Reagent 6A,6B&6C** AEC requires mixing! (10min)				
Step 12	Counter stain Hematoxylin User supplied				
Step 13	**Reagent 6** Simpo-Mount (RTU) Do not coverslip!				
Result	Stain pattern on controls is correct: Fill in Yes or NO				

To Coverslip see protocol note 3.

9620 Medical Center Drive, Suite 200, Rockville, MD 20850, USA Phone: 1-301-340-3188 Fax: 301-340-9254