Polink DS-MR-Ms B Kit for Immunohistochemistry Staining

Polymer-HRP & AP double staining kit to detect a rabbit and a mouse primary antibody on mouse tissue with BCIP/NBT (Purple) and AEC (Red).

Storage: 2-8ºC

Catalog No.:
- ☐ DS233B-6 — 12ml* — 120 slides**
- ☐ DS233B-18 — 36ml* — 360 slides**
- ☐ DS233B-60 — 120ml* — 1200slides**

**Total volume of polymer Conjugates*

*** if use 100µl per slide*

Intended Use:

The **Polink DS-MR-Ms B Kit** is designed to use with user supplied mouse and rabbit primary antibody to detect two distinct antigens on mouse tissue or cell samples. DS233 kits can be used on frozen or paraffin embedded tissues, and freshly prepared monolayer cell smears. Our system is designed not give background on most mouse strains however there may be some mouse strains especially when using frozen that require additional blocking; we recommend GBI's Klear Mouse Block (D54-xx) to improve specificity of the mouse primary antibody on mouse tissue.

Double staining is one of most common methods used in immunohistostaining that allows for revealing two distinct antigens in a single tissue[1, 2]. **Polink DS-MR-Ms B Kit** from GBI Labs-Inc supplies two polymer enzyme conjugates: Mouse AP Polymer and Rabbit HRP Polymer with two distinct substrates/chromogens, BCIP/NBT (purple color, use with the Mouse AP Polymer) and AEC (red color, use with the Rabbit HRP Polymer). A Primer step is used to increase specificity of antibody staining. Both enzyme conjugates are applied to the specimen at the same time and mixed on the slide. This kit offers simplified steps that make for a quicker and easier protocol than that used in a sequential procedure. **Polink DS-MR-Ms B Kit** is non-biotin system that avoids endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	12ml Kit	36ml Kit	120ml Kit
Reagent 1	Mouse Primer (RTU)	12ml	18mlx2	120ml
Reagent 2	Mouse AP Polymer (RTU)	6ml	18ml	60ml
Reagent 3	Rabbit HRP Polymer (RTU)	6ml	18ml	60ml
Reagent 4	BCIP/NBT Chromogen(RTU)	15ml	18mlx2	120ml
Reagent 5A	AEC Substrate (20x)	1ml	2ml	6ml
Reagent 5B	AEC Chromogen (20x)	2ml	4ml	12ml
Reagent 5C	Hydrogen Peroxide (20x)	1ml	2ml	6ml
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)

Reagent	Staining Procedure	Incubation Time (Min.)
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.	10 min.
2. HIER Pretreatment: Refer to Ab 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. **Klear Mouse Block A (D54-A)**	a. Add 2 drops (100µl) or enough volume of **D54-A Klear Ms**	30 min.

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

Not provided **(optional see protocol note 2)**	**Blocking A** to cover the tissue section and Incubate. b. Rinse with PBS with 0.05% Tween-20 for 2 min., 3 times.	
4. **Klear Mouse Block B (D54-B)** Not provided **(optional see protocol note 2)**	a. Add 2 drops (100µl) or enough volume of **D54-B Klear Ms Blocking B** to cover the tissue section and Incubate. **Do not** exceed 5min. b. Rinse with PBS with 0.05% Tween-20 for 2 min., 3 times.	5 min.
5. Mouse antibody 1 and Rabbit antibody 2: Supplied by user	**Note:** Investigator needs to optimize titer of primary antibody and incubation time prior to double staining. a. Apply 2 drops or enough volume of both Mouse primary Antibody 1 and Rabbit primary Antibody 2 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.
6. **Reagent 1** Mouse Primer (RTU)	a. Add 1-2 drops (100µl) or enough volume of **Reagent 1** (Mouse Primer) to cover the tissue section and Incubate Room Temperature for 10-15minutes. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	10-15min
7. Mix **Reagent 2&3** **Reagent 2 :** Mouse AP Polymer (RTU) **Reagent 3:** Rabbit HRP Polymer (RTU)	**Note:** Make sufficient polymer mixture by adding **Reagent 2** (Mouse AP Polymer) and **Reagent 3** (Rabbit HRP Polymer) at 1:1 ratio, mix well. Do Not mix more than you need for the experiment because the polymer mixture is not stable for long term storage. a. Apply 1 to 2 drops (50-100µl) of the mixture to cover the tissue completely. b. Incubate in moist chamber for 30 min. c. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	30 min.
8. **Reagent 4** BCIP/NBT Chromogen (RTU)	a. Apply 2 drops or enough volume of **Reagent 4** (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.
9. **Reagent 5A, 5B, 5C:** **5A:**AEC Substrate Buffer (20x) **5B:**AEC Chromogen (20x) **5C:**Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of **Reagent 5A** to 1ml distill water. Mix well . Add 2 drops of **Reagent 5B** and 1 drop of **Reagent 5C** 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
10. 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.	
11. **Reagent 6:** Simpo-Mount (RTU) **To coverslip see protocol note 3**.	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. 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. **Klear Mouse Block** (sample provided) the anti-mouse secondary has been absorbed to rat serum resulting in most mouse strains having no background, however some mouse strains may need additional blocking. **Klear Mouse Block (D54-xx)** works very well on frozen tissue.
3. Simpo-Mount is an aqueous-based mounting media 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: Please wear gloves and take other necessary precautions.

Remarks: For research use only.

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 DS233B Kit

We designed this work sheet to help you keep track of each step. We recommend you use this sheet to record the actual time of each step conducted as it will be helpful for questions with our technical support.

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

Step/ Protocol	Protocol DS233B	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase Block				
Step 2	HIER if needed				
Step 3 Optional	Klear Mouse Block A (D54-A) 30min				
Step 4 Optional	Klear Mouse Block B (D54-B) 5min				
Step 5	Ms 1°Ab & Rb 1°Ab mix (30-60 min.)				
Step 6	**Reagent 1** Mouse Primer (15 min.)				
Step 7	**Reagent 2 & Reagent 3** Ms AP Polymer & Rb HRP Polymer require mixing (30 min.)				
Step 8	**Reagent 4** BCIP/NBT (3-10 min.)				
Step 9	**Reagent 5A, 5B, & 5C** AEC Requires mixing! (5-15 min.)				
Step 10	Counter stain Hematoxylin User supplied				
Step 11	**Reagent 6** Simpo Mount Do not coverslip!				

Testing result:

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