Polink DS-RR-Hu/Ms B Kit for Immunohistochemistry Staining

Polymer-HRP & AP double staining kit to detect two rabbit primary antibodies on human/mouse tissue with BCIP(Purple) and AEC(Red)

Storage: 2-8ºC

Catalog No.:
- ☐ DS204B-6/D81-6 12mL* 60 slides**
- ☐ DS204B-18 36mL* 180 slides**
- ☐ DS204B-60 120mL* 600slides**

**Total volume of polymer Conjugates*

*** if use 100µL per slide*

Intended Use:

The **Polink DS-RR-Hu/Ms B Kit** is designed to use with user supplied two rabbit antibodies to detect two distinct antigens on human tissue or cell samples. This kit has been tested in paraffin tissue. However, this kit can be used on frozen specimen and freshly prepared monolayer cell smears.

Double staining is one of most common methods used in immunohistostaining that allow revealing two distinct antigens in a single tissue [1, 2]. **Polink DS-RR-Hu/Ms B Kit** from GBI Labs (Golden Bridge International) supplies two polymer enzyme conjugates: HRP polymer anti-Rabbit IgG and AP polymer anti-Rabbit IgG with two distinct substrates/chromogens, AEC (Red color, use with HRP polymer anti-Rabbit IgG) and BCIP/NBT (Purple/Blue color, use with AP polymer anti-Rabbit IgG). **Polink DS-RR-Hu/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	Rabbit AP polymer (RTU)	6mL	18mL	60mL
Reagent 2	BCIP/NBT (RTU)	6mL	18mL	60mL
Reagent 3A	DS-RR Blocker A	6mL	18mL	60mL
Reagent 3B	DS-RR Blocker B	6mL	18mL	60mL
Reagent 4	Rabbit HRP(AEC) Polymer (RTU)	6mL	18mL	60mL
Reagent 5A	AEC Substrate Buffer (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)	7mL	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 need to be adhered to the slide tightly to avoid tissue falling off.
3. Paraffin embedded section must be deparffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made as much 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. It takes about 30 minutes to dissolve Fast Red tablet into the substrate buffer. Make sure to start preparing Fast Red solution near the end of the secondary antibody incubation.
7. Proceed with IHC staining: DO NOT let specimen or tissue dry from this point on.
8. **Note:** 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 slides using 2 changes of distilled water.	10min
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 8**	

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	above); 3 times for 2 minutes each.	
3. Preblock (optional)	a. For paraffin section, Improved formula saves the need for a preblock step. b. For frozen tissue, preblock may or may not be required depending on fixative. (Preblock catalogue No.:E07 was Recommended.)	
4. Rabbit Antibody 1: Supplied by user	***Notes:*** Investigator needs to optimize dilution and incubation times prior to double staining. a. Apply 2 drops or enough volume of rabbit primary antibody 1 to cover the tissue completely. 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
5.**Reagent 1:** Rabbit AP Polymer (RTU)	a. Apply 2 drops or enough volume of **Reagent 1** Rabbit AP Polymer to cover each section. b. Incubate in moist chamber for 20-30 min. c. Wash with **1X TBS-T**; 3 times for 2 minutes each.	20-30 min
6. **Reagents 2:** BCIP/NBT (RTU)	a. Apply 2 drops or enough volume of **Reagents 2** 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
7. **Reagent 3A:** DS-RR Blocker A	a. Apply 2 drops or enough volume of **Reagent 3A** DS-RR Blocker A to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 30 min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30 min
8. **Reagent 3B:** DS-RR Blocker B	a. Apply 2 drops or enough volume of **Reagent 3B** DS-RR Blocker B to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 5 min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	5 min
9. Rabbit antibody 2: Supplied by user	***Notes:*** Investigator needs to optimize dilution and incubation times prior to double staining. a. Apply 2 drops or enough volume of rabbit primary antibody 2 to cover the tissue completely. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30-60 min
10. **Reagent 4:** Rabbit HRP(AEC) Polymer (RTU)	a. Apply 2 drops or enough volume of **Reagent 4** Rabbit HRP(AEC) Polymer to cover each section. b. Incubate in moist chamber for 20-30 min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	20-30 min
11. **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** and 1 drop or **2 drops** (for higher sensitivity and contrast) of reagent **5B** and 1 drop of Reagent **5C** 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 5-10 min, observe appropriate color development. c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate.**)	5-10 min
12. Counterstain (**Optional**) Not provided	a. Counterstain with 2 drops (100 µl) or enough volume of counterstain solution to completely cover tissue. Incubate for 10-15 seconds. b. Rinse thoroughly with tap water for 2-3 min. c. Rinse well in distilled water.	
13. **Reagent 6:** Simpo-Mount	a. Apply 2 drops (100 µl) or enough volume **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 effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.

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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.

Precautious:
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 Immunocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997

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