

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: 2-8°C

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 | AP polymer anti-Rabbit IgG (RTU) | 6ml | 18ml | 60ml |
| Reagent 2 | BCIP/NBT Solution(RTU) | 6ml | 18ml | 60ml |
| Reagent 3A | DS-RR Blocker A | 6ml | 18ml | 60ml |
| Reagent 3B | DS-RR Blocker B | 6ml | 18ml | 60ml |
| Reagent 4 | HRP polymer anti Rabbit-IgG (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 | Simplo-Mount solution (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 need to be adhered to the slide tightly to avoid tissue falling off.
3. Paraffin embedded section must be deparaffinized 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 IHC staining: DO NOT let specimen or tissue dry from this point on.

| Reagent | Staining Procedure | Incubation Time (Min.) |
|--|---|------------------------|
| 1. Peroxidase Blocking Reagent Not provided | a. Incubate slides in peroxidase blocking reagent (Ready-to-use 3% H ₂ O ₂ solution) for 10 minutes. b. Rinse the slide using distilled water. | 10 min |
| 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 for 2 min., 3 times. | |
| 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 | |

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| | 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. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 30-60 min |
| 5. Reagent 1: AP polymer anti-Rabbit IgG (RTU) | a. Apply 2 drops or enough volume of Reagent 1 AP polymer anti-Rabbit IgG to cover each section. b. Incubate in moist chamber for 20-30 min. c. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 20-30 min |
| 6. 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 3-10 min. b. Rinse thoroughly with distilled water. c. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 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. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 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. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 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. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 30-60 min |
| 10. Reagent 4: HRP polymer anti Rabbit-IgG (RTU) | a. Apply 2 drops or enough volume of Reagent 4 HRP polymer anti Rabbit-IgG to cover each section. b. Incubate in moist chamber for 20-30 min. c. Rinse with PBS containing 0.05% Tween-20 for 2 min., 3 times. | 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 (100 µl) 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: Simpomount | 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.
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.

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