# Polink DS-MR-Hu B1 Kit

(Polymer-HRP and AP Kit)
(Detects Mouse and Rabbit primary antibodies for human tissue with BCIP/NBT(Purple) and AEC(Red))

Storage: 2-8ºC

Catalog No.:

| | | | |
|---|---|---|---|
| ☐ | DS201B-6/(D63-6) | 6mL* | 60 slides** |
| ☐ | DS201B-18 | 18mL* | 180 slides** |
| ☐ | DS201B-60 | 60mL* | 600 slides** |

**Total volume of polymer Conjugates*
*** if use 100µl per slide*

**Intended Use:**
The **Polink DS-MR-Hu B1 Kit** is designed to use with user supplied mouse and 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 the most common methods used in immunohistochemistry to screen two distinct antigens in a single tissue [1, 2]. GBI Labs **Polink DS-MR-Hu B1 Kit** supplies user with two polymer enzyme conjugates; an HRP-Polymer anti-Mouse IgG and AP-Polymer anti-Rabbit IgG with reactive chromogens for each enzyme. The AEC chromogen (Red Brick color) is used with HRP-Polymer anti-Mouse IgG and BCIP/NBT (Purple/Blue color) is used with AP-Polymer anti-Rabbit IgG. Simplified steps offer a much faster protocol as the enzyme conjugates are applied to the specimen as a mixture. Both the enzyme conjugated polymers and chromogens are optimized to give the strongest signal with no background. **Polink DS-MR-Hu B1 Kit** is non-biotin system that avoids the need to block endogenous biotin causing non-specific binding.

**Kit Components:**

| Component No. | Content | DS201B-6 | DS201B-18 | DS201B-60 |
|---|---|---|---|---|
| **Reagent 1** | Mouse HRP Polymer (RTU) | 6mL | 18mL | 60mL |
| **Reagent 2** | Rabbit AP Polymer (RTU) | 6mL | 18mL | 60mL |
| **Reagent 3** | BCIP/NBT (RTU) | 6mL | 18mL | 60mL |
| **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) | 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. Tissues 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. 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 pH 7.6. 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 E36.** It is fast, easy, and it will block endogenous alkaline phosphatase<br>a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent.<br>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. Follow the recommendations of the antibody vendor.<br>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. Pre-Block (optional): | For paraffin section, Improved formula saves the need for a pre-block step.<br>For frozen tissue, pre-block may or may not be required depending on fixative.<br>(Pre-block catalogue No.: E07 is recommended). | |



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|---|---|---|
| 4. Mouse antibody 1 and Rabbit antibody 2: Supplied by user | ***Notes:*** Investigator needs to optimize dilution and incubation times prior to double staining.<br>a. Apply 2 drops or enough volume of both Primary Antibody 1and Antibody 2 to cover the tissue completely. Mix well on the slide and incubate in moist chamber for 30-60 min.<br>b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each. | 30-60 min |
| 5. **Reagents 1:** Mouse HRP Polymer (RTU) | a. Apply 1 to 2 drops (50-100µL) of **Reagent 1 (**Mouse HRP Polymer) to cover each section.<br>b. Incubate in moist chamber for 15-30 min.<br>c. Wash with **1X TBS-T only**; 3 times for 30 seconds to 2 minutes each.<br>**Note:** longer incubation may increase background | 15-30 min |
| 6. **Reagents 2:** Rabbit AP Polymer (RTU) | a. Apply 1 to 2 drops of **Reagent 2 (**Rabbit AP Polymer) to cover each section<br>b. Incubate in moist chamber for 15-30 min.<br>c. Wash with **1X TBS-T only**; 3 times for 30 seconds to 2 minutes each.<br>**Note:** longer incubation may increase background | 15-30 min |
| 7. **Reagent 3:** BCIP/NBT (RTU) | a. Apply 2 drops or enough volume of **Reagent 3** (BCIP/NBT) to completely cover tissue.<br>b. Incubate for 3-10 min.<br>c. Rinse thoroughly with distilled water.<br>d. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each. | 5-10 min |
| 8. **Reagents 4A, 4B, 4C:**<br>**Reagent 4A:** AEC Substrate (20x)<br>**Reagent 4B:** AEC Chromogen (20x)<br>**Reagent 4C:** Hydrogen Peroxide (20x) | a. Add 1 drop (50µL) of **Reagent 4A** to 1mL distilled water. Mix well. Add 2 drops of **Reagent 4B** and 1 drop of **Reagent 4C** to diluted reagent 1. Mix well. Keep away from light and use within 1 hour.<br>b. Apply 2 drops (100µL) or enough volume of pre-mixed AEC solution to completely cover the tissue. Incubate for 5-15min, observe appropriate color development.<br>c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate.** ) | 10 min |
| 9. HEMATOXYLIN: Not provided | a. Counterstain with 2 drops (100µL) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds.<br>b. Rinse thoroughly with tap water for 2-3 min.<br>c. Put slides in PBS until show blue color (about ½ - 1 min.)<br>d. Rinse well in distilled water. | |
| 10. **Reagent 5:** Simpo-Mount (RTU) | a. Apply 2 drops (100µL) or enough volume **Reagent 5** to cover tissue when tissue is wet. Rotate the slides to allow Simpo-Mount to spread evenly. DO NOT coverslip.<br>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<br>OR<br>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 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.

**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



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