Polink DS-MR-Hu B2 Kit for for Immunohistochemistry Staining

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

Storage: 4-8ºC

Catalog No.: ☐ DS202B-6/D64-6 12ml* 120 slides**
☐ DS202B-18 36ml* 360 slides**
☐ DS202B-60 120ml* 1200 slides**

**Volume of polymer conjugate*
*** If use 100µl per slide*

Intended Use:

The **Polink DS-MR-Hu B2 Kit** is designed to use with user supplied mouse antibody and rabbit antibody 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-MR-Hu B2 Kit** from GBI Labs (Golden Bridge International) supplies two polymer enzyme conjugates: HRP-Polymer anti Rabbit IgG and AP-Polymer anti Mouse 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 Mouse IgG). User may apply the two enzyme conjugates onto the specimen at the same time and mix them on the slide. Simplified steps offer user much faster and quicker protocol than a sequential procedure. **Polink DS-MR-Hu B2 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	HRP-Polymer anti Rabbit IgG (RTU)	6ml	18ml	60ml
Reagent 2	AP- Polymer anti Mouse IgG (RTU)	6ml	18ml	60ml
Reagent 3	BCIP/NBT Solution (RTU)	12ml	18mlx2	120ml
Reagent 4A	AEC Substrate Buffer (20x)	1ml	2ml	6ml
Reagent 4B	AEC Chromogen (20x)	2ml	4ml	12ml
Reagent 4C	Hydrogen Peroxide (20x)	1ml	2ml	6ml
Reagent 5	Simpo-Mount	12ml	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 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. 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.	10min
2. HIER Pretreatment: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor.	

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	b. 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. Preblock (optional)	For paraffin section, Improved formula saves the need for a preblock step. For frozen tissue, preblock may or may not be required depending on fixative. (Preblock catalogue No.:E07 was Recommended.)	
4. Mouse antibody 1 and Rabbit antibody 2: Supplied by user	**Note:** Investigator needs to optimize dilution prior to triple staining. a. Apply 2 drops or enough volume of mouse and rabbit primary antibody mixture to cover the tissue completely. Incubate in moist chamber for 30-60 min. Recommend 30min to shorten total protocol time. 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 and 2 Reagent 1:** HRP-Polymer anti Rabbit IgG (RTU) **Reagent 2:** AP- Polymer anti Mouse IgG (RTU)	**Note:** Make sufficient polymer mixture by adding **Reagent 1** (HRP-Polymer anti Rabbit IgG) and **Reagent 2** (AP- Polymer anti Mouse IgG) at 1:1 ratio, mix well. Do Not Mix More than you need for the experiment because the polymer mixture may not be as stable as non-mixed polymer. 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
6. **Reagents 3** BCIP/NBT Chromogen (RTU)	a. Apply 2 drops or enough volume of **Reagents 3** BCIP/NBT CHROMOGEN to completely cover tissue. Incubate for 5-10 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.	5-10 min
7. **Reagent 4A, 4B, 4C** **Reagent 4A:** AEC Substrate Buffer (20x) **Reagent 4B:** AEC Chromogen (20x) **Reagent 4C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of **Reagent 4A** and 1 drop or **2 drops** (for higher sensitivity and contrast) of **Reagent 4B** and 1 drop of **Reagent 4C** 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 10 min, observe appropriate color development. c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate.**)	10 min
8. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100 µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds. b. Rinse thoroughly with tap water for 2 min. c. Put slides in PBS until show blue color (about ½ - 1 min.) d. Rinse well in distilled water.	
9. **Reagent 5:** Simpo-Mount	a. Apply 2 drops (100 µl) or enough volume **Reagent 5** 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.

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.

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2. Polak J. M and Van Noorden S. *Introduction to Immnocytochemistry Second Edition*. Bios Scientific Publishers. P41-54. 1997

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