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

Polymer HRP and AP Double Staining Kit for Rabbit & Rat Primary Antibodies on Human and Mouse Tissue with BCIP/NBT(Purple) and AEC(Red)

Storage: 2-8ºC

Catalog No.:
- ☐ DS211B-6 12mL* for 120 slides**
- ☐ DS211B-18 36mL* for 360 slides**
- ☐ DS211B-60 120mL* for 1200 slides**

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

Intended Use:

The **Polink DS-RRt-Hu/Ms B Kit** is designed for use with user supplied rabbit and rat primary antibodies to detect two distinct antigens on human and mouse tissue or cell samples. **Polink DS-RRt-Hu/Ms B Kit** can be developed for frozen or paraffin embedded tissue, or freshly prepared monolayer cell smears.

Double staining is a common method used in immunohistostaining, allowing for the detection of two distinct antigens in a single tissue. **Polink DS-RRt-Hu/Ms B Kit** from GBI labs supplies the user with two polymer enzyme conjugates: HRP polymer anti-Rat IgG (minimal cross reaction to mouse) and AP polymer anti-Rabbit IgG with two distinct substrates/chromogens, BCIP/NBT and AEC. AEC chromogen reacts with the anti-Rat HRP polymer conjugate to produce a red color. BCIP/NBT reacts with anti-Rabbit AP polymer to produce the subsequent purple color. **Polink DS-RRt-Hu/Ms B Kit** is a non-biotin system avoiding the extra steps involved in blocking non-specific binding due to endogenous biotin.

Kit Components:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Rat (No Ms) HRP(AEC) Polymer (RTU)	6mL	18mL	60mL
Reagent 2	Rabbit AP Polymer (RTU)	6mL	18mL	60mL
Reagent 3	BCIP/NBT (RTU)	15mL	18mLx2	120mL
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)	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 prepared as close to a monolayer as possible to obtain satisfactory results.
5. Three control slides are recommended for interpretation of results: positive, reagent (slides treated with Isotype control reagent), and negative control.
6. Proceed with IHC staining: **DO NOT** let specimen or tissue dry from this point on.
7. 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 interpreting results.
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)

Equipment or material needed but not provided:

1. Equipment and material for deparaffinization, such as fume absorbing hood, etc.
2. Heat source (microwave or hot plate) for HIER and antigen retrieval buffers.
3. Thermometer
4. Beaker
5. Timer
6. Wash buffer: 0.01 M PBS with 0.5% Tween20, pH7.4
7. Peroxidase and alkaline phosphatase blocking buffer
8. 100% ethanol
9. 100% Xylene
10. Hematoxylin

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Steps / 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 above)**; 3 times for 2 minutes each.	Up to 1 hour
3. Primary Antibody Mix: **one Rat and one Rabbit antibody** Supplied by user	**Note:** Investigator needs to optimize primary antibody titer prior to double staining. a. Apply 2 drops or enough volume of rat 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.	30-60min
4. Mix **Reagent 1& Reagent 2** **Reagent 1** Rat (No Ms) HRP(AEC) Polymer (RTU) **Reagent 2** Rabbit AP Polymer (RTU)	**Note:** Make sufficient polymer mixture by adding **Reagent 1** Rat (No Ms) HRP(AEC) Polymer and **Reagent 2** (Rabbit AP Polymer) at 1:1 ratio, mix well. a. Apply 1 to 2 drops (50-100µl) of the mixture to cover each section. b. Incubate in moist chamber for 30 min. c. Wash with **1X TBS-T only**; 3 times for 2 minutes each. **Make enough mixture for the experiment. Do not make extra volume as mixture is not stable .**	30 min.
5. **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 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
6. **Reagents 4A, 4B, 4C** **4A:** AEC Substrate (20x) **4B**: AEC Chromogen (20x**)** **4C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µl) of **Reagents 4A** and 1 drop or **2 drops** (for higher sensitivity and contrast) **Reagents 4B** and 1 drop of **Reagents 4C** to 1mL distill water. Mix well. Keep away from light and use within 1 hour. b. Apply 2 drops (100µl) or enough volume of AEC chromogen working 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
7.Counterstain Hematoxylin (Not provided)	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to completely cover tissue. Incubate for 5 to 10 seconds. **DO NOT** over stain with hematoxylin! b. Wash slides thoroughly with tap water for 1 minute. c. Put slides in PBS for 5-10 seconds to blue, **DO NOT** over blue. d. Wash slides well in distilled or tap water for 1 minute.	5 seconds
8. **Reagent 5:** Simpo-Mount (RTU) **To coverslip see protocol note 3**.	a. Apply 2 drops (100µl) or enough volume of **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

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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.

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 Immnocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997

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Work Sheet for DS211B Kit

We designed these work sheets to help you track of each step. When staining fails these sheets help our technical support staff to pinpoint the problem.
To insure that all steps are done properly, we recommend that the user fill in the actual time of their experimental step and any variation. Results will vary if time recommendations are not followed. RTU translates to ready to use.

- 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 DS211B	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase & levamisole Block E36 is recommended. User supplied				
Step 2	HIER if needed				
Step 3	Rb 1°Ab & Rat 1°Ab mix (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** Rat(no Ms) HRP(AEC) Polymer & Rabbit AP Polymer require mixing (30 min.)				
Step 5	**Reagent 3** BCIP/NBT RTU (10min)				
Step 6	**Reagent 4A, Reagent 4B& Reagent 4C** AEC requires mixing! (10min)				
Step 7	Counter stain Hematoxylin User supplied				
Step 8	**Reagent 5** Simpo-Mount (RTU) **To coverslip see protocol note 3**.				
Result	**Stain pattern on controls are correct: Fill in Yes or No**				

Testing result:

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