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

Polymer-HRP and AP Kit to Detect Goat and Rabbit Primary Antibodies for Human or Mouse Tissue with BCIP/NBT (Purple) and AEC (Red)

Storage: 2-8ºC

Catalog No.:

☐	DS205B-6	12mL*	60 slides**
☐	DS205B -18	36mL*	180 slides**
☐	DS205B -60	120mL*	600 slides**

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

Intended Use:
Polink DS-GR-Hu/Ms B Kit is designed for use with two user-supplied primary antibodies, one goat and one rabbit, to detect two distinct antigens on human and mouse 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 commonly used methods in immunohistostaining for revealing two distinct antigens in a single tissue[1, 2]. **Polink DS-GR-Hu/Ms B Kit** from GBI Labs (Golden Bridge International) supplies two polymer enzyme conjugates: AP Polymer anti-Goat IgG and HRP-AEC Polymer anti-Rabbit IgG with two substrates/chromogens, BCIP/NBT (Purple/Blue color, use with AP Polymer anti-Goat IgG) and AEC (Red color, use with HRP-AEC Polymer anti-Rabbit IgG). **Polink DS-GR-Hu/Ms B Kit** is a non-biotin system, avoiding blocking steps for endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	6mL Kit	36mL Kit	120mL Kit
Reagent 1	Goat AP Polymer (RTU)	6mL	18mL	60mL
Reagent 2	DS-GR Blocker (RTU)	6mL	18mL	60mL
Reagent 3	Rabbit HRP-AEC Polymer (RTU)	6mL	18mL	60mL
Reagent 4	BCIP/NBT Chromogen(RTU)	7mL	18mL	70mL
Reagent 5A	AEC Substrate Buffer (20x)	1mL	1mL	3mL
Reagent 5B	AEC Chromogen (20x)	2mL	2mL	6mL
Reagent 5C	Hydrogen Peroxide (20x)	1mL	1mL	3mL
Reagent 6	Simpo-Mount (RTU)	7mL	18mL	70mL

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 deparffinized with xylene and rehydrated with a graded series of alcohols 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. To maximize the difference between hemotoxylin counter stain (blue) and BCIP/NBT (purple) shorten the time in counter stain and do not blue with pH buffer.
7. DO NOT let specimen or tissue dry during protocol.
8. 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 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.	
3. Primary Antibody Mix: **one**	**Note:** Investigator needs to optimize dilution prior to double staining.	30-60min

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Goat and one Rabbit antibody Supplied by user	a. Apply 2drops (100µL) or enough volume of goat 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/0.05% Tween20 for 2 minutes, 3 times.	
4.**Reagent 1:** Goat AP Polymer (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 1** (Goat AP Polymer) to cover each section. b. Incubate in moist chamber for 15 min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	15min
5. **Reagent 2:** DS- GR-Blocker(RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 2 (**DS-GR-Blocker) to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 10 min. b. Tap off liquid and wipe excess do not let dry. Rinse with PBS-T containing 0.05% Tween-20 or **1X TBS-T**.	10min
6. **Reagent 3:** Rabbit HRP-AEC Polymer (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 3** (Rabbit HRP-AEC Polymer) to cover each section. b. Incubate in moist chamber for 15 min. c. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	15min
7. **Reagents 4:** BCIP/NBT Chromogen(RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 4** (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.
8. **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** to 1mL distill water. Mix well . Add 2 drops of **Reagent 5B** and 1 drop of **Reagent 5C** to diluted AEC Substrate. Mix well. Keep away from light and use within 1 hour. b. Apply 2 drops (100µL) or enough volume of AEC working solution to completely cover the tissue. Incubate for 5-15 min, observe appropriate color development. c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate.**)	5-15 min
9. Counterstain (**Optional**) Not provided	a. Counterstain with 2 drops (100µL) 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	
10. **Reagent 6:** Simpo-Mount (RTU)	a. Apply 2 drops (100µL) or enough volume of **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 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, eye protection and take other necessary precautions. If any of the reagent come in contact with skin wash area completely with plenty of water and soap. If irritation develops seek medical attention.

Remarks:

This kit is 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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Work Sheet for DS205B Kit

We designed this work sheet to help you keep track of each step. We recommend you use this sheet to record the actual time of each step conducted as it will be helpful for questions with our technical support.

- 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 DS205B	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase Block				
Step 2	HIER if needed				
Step 3	Gt 1°Ab & Rb 1°Ab mix (30-60 min.)				
Step 4	**Reagent 1** Goat AP Polymer (RTU) 15min				
Step 5	**Reagent 2** DS-GR Blocker (RTU) 10 min				
Step 6	**Reagent 3** Rabbit HRP-AEC Polymer (RTU) 15min				
Step 7	**Reagent 4** BCIP/NBT Chromogen (RTU) 3-10min				
Step 8	**Reagent 5A, 5B & 5C** AEC Requires mixing! (5-15 min.)				
Step 9	Counter stain Hematoxylin User supplied				
Step 10	**Reagent 6** Simpo-Mount (RTU) Do not coverslip!				

Testing result:

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