Polink DS-GM-Hu B Kit for Immunohistochemistry Staining

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

Storage: 2-8ºC

Catalog No.:

☐	DS207B-6	12mL*	60 slides**
☐	DS207B-18	36mL*	180 slides**
☐	DS207B-60	120mL*	600 slides**

**Total volume of polymer Conjugates*

*** If use 100µl per slide*

Intended Use:

The **Polink DS-GM-Hu B Kit** is designed to use with user supplied goat and mouse 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 allows for revealing two distinct antigens in a single tissue[1, 2]. The **Polink DS-GM-Hu B Kit** from GBI Labs (Golden Bridge International) supplies two polymer enzyme conjugates: AP polymer anti-Goat IgG and HRP(AEC) polymer anti-Mouse IgG with two distinct substrates/chromogens, BCIP/NBT (Purple) and AEC (Red). User will apply the two enzyme conjugates onto the specimen sequentially. If only the anti-goat antigen is present only the BCIP/NBT(Purple) will be present and if the mouse antigen is present only the AEC(Red) will be present. The **Polink DS-GM-Hu B Kit** is non-biotin system avoiding endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Goat AP Polymer (RTU)	6mL	18mL	60mL
Reagent 2	DS-GM Blocker (RTU)	6mL	18mL	60mL
Reagent 3	Mouse HRP(AEC) Polymer (RTU)	6mL	18mL	60mL
Reagent 4	BCIP/NBT (RTU)	7mL	18mL	120mL
Reagent 5A	AEC Substrate (20x)	1mL	2mL	4mL
Reagent 5B	AEC Chromogen (20x)	2mL	4mL	8mL
Reagent 5C	Hydrogen Peroxide (20x)	1mL	2mL	4mL
Reagent 6	Simpo-Mount (RTU)	7mL	18mL	70mL

Gt=Goat Ms=Mouse

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 must be adhered to the slide properly to ensure maximum quality staining.
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 made up to as much of a monolayer as possible to obtain satisfactory results.
5. Three control slides will aid the interpretation of the result: positive and negative tissue controls, reagent control (slides treated with Isotype control reagent).
6. Proceed with IHC staining: **DO NOT** let specimens or tissues dry from this point on.
7. 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 results.

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
1. Peroxidase and alkaline phosphatase Blocking Reagent Supplied by user	a. Incubate slides in peroxidase and alkaline phosphatase blocking reagent (Klear Dual Enzyme Block E36 is Recommended) for 10 minutes. 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. Refer to antibody datasheet. b. Wash with PBS/0.05% Tween20 for 2 minutes, 3 times.	Up to 1 hour
3. Primary Antibody Mix: **one Goat and one Mouse antibodies** Supplied by user	**Note:** Investigator needs to optimize dilution prior to double staining. a. Apply 2 drops or enough volume of goat and mouse 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.	30-60min
4. **Reagent 1** Goat AP Polymer (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 1** (Goat AP Polymer) to cover each section. b. Incubate in moist chamber for 15 min. c. Wash with PBS/ 0.05% Tween20 for 2 minutes, 3 times.	15min
5. **Reagent 2** DS-GM Blocker (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 2** (DS-GM Blocker) to cover each section. b. Incubate in moist chamber for 10 min. c. Blot off solution. Rinse 1x with PBS (5sec)	10min
6**. Reagent 3** Mouse HRP(AEC) Polymer (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 3** (Mouse HRP(AEC) Polymer) to cover each section. b. Incubate in moist chamber for 15 min. c. Wash with PBS/ 0.05% Tween20 for 2 minutes, 3 times. d. Rinse with tap water.	15min
7. **Reagent 4:** BCIP/NBT (RTU)	a. Apply 2 drops or enough volume of **Reagent 4** (BCIP/NBT) to completely cover tissue. Incubate for 5-10 min. b. Rinse thoroughly with distilled water.	5-10min
8. **Reagent 5A, 5B, 5C:** **Reagent 5A:** AEC Substrate (20x) **Reagent 5B:** AEC Chromogen (20x) **Reagent 5C:** Hydrogen Peroxide (20x)	a. Add 1 drop (50µL) of **Reagent 5A** to 1mL distilled water. Mix well . Add 2 drops of **Reagent 5B** and 1 drop of **Reagent 5C** to diluted reagent 1. Mix well. Keep away from light and use within 1 hour. 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. c. Rinse well with distilled water. (**AEC is alcohol soluble; do not dehydrate.**)	10min
9. HEMATOXYLIN Not provided	a. Counterstain with 2 drops (100µL) or enough volume of hematoxylin to completely cover tissue. Incubate for 10-15 seconds. b. Rinse thoroughly with tap water for 2-3 min. c. Put slides in PBS until show blue color (about ½ - 1 min.) d. 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.	30min. 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:
Standard laboratory personal protective equipment should be worn: i.e. gloves, eye protection and appropriate lab coat.

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 DS207B 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

Protocol Step	DS207A Protocol Reagent/Time	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase & levamisole Block E36 is recommended. User supplied				
Step 2 Optional	HIER if needed User supplied (up to 60 min)				
Step 3	Mix one Goat and one Mouse primary antibodies User supplied (30-60 min)				
Step 4	**Reagent 1** Goat AP Polymer RTU (15min)				
Step 5	**Reagent 2** DS-GM Blocker RTU (10min) Rinse with PBS then Go immediately to step 6				
Step 6	**Reagent3** Mouse HRP(AEC) Polymer RTU (15min)				
Step 7	**Reagent 4** BCIP/NBT RTU (10min)				
Step 8	**Reagent 5A, 5B & 5C** AEC requires mixing (10min)				
Step 9	Counter stain Hematoxylin User supplied				
Step 10	**Reagent 6** Simpo-Mount RTU Do not coverslip!				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

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