Polink DS-GM-Hu A Kit for Immunohistochemistry Staining

Polymer-HRP and AP Kit to Detect Goat and Mouse Primary Antibodies for Human Tissue Screens with DAB (Brown) and GBI-Permanent Red (Red)

Storage: 2-8ºC

Cat No.:
- ☐ DS207A-6 12mL* 60 slides**
- ☐ DS207A-18 36mL* 180 slides**
- ☐ DS207A-60 120mL* 600 slides**

**Total volume of polymer Conjugate*
*** if use 100µL per slide*

Intended Use:

The **Polink DS-GM-Hu A 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 a common method used in immunohistochemistry for the detection of two distinct antigens in a single tissue [1, 2]. The **Polink DS-GM-Hu A Kit** from GBI Labs (Golden Bridge International) supplies two polymer enzyme conjugates: AP polymer anti-Goat IgG and HRP polymer anti-Mouse IgG with two distinct substrates/chromogens, DAB(Brown) and GBI Permanent Red(Red). Simplified steps offer a convenient protocol as the enzyme conjugates are applied to the specimen sequentially. When the anti-goat antigen is present only the GBI Permanent Red will be present or when anti-mouse antigen is present only the DAB(brown) will be present. The **Polink DS-GM-Hu A 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)	6 mL	18mL	60mL
Reagent 2A	GBI-Permanent Red Substrate (RTU)	15mL	18mLx2	70mL
Reagent 2B	GBI-Permanent Red Activator (5x)	3mL	7.2mL	14mL
Reagent 2C	GBI-Permanent Red Chromogen (100x)	150µL	360µL	0.7mL
Reagent 3	DS-GM Blocker (RTU)	6 mL	18mL	60mL
Reagent 4	Mouse HRP Polymer (RTU)	6 mL	18mL	60mL
Reagent 5A	DAB Substrate(RTU)	12mL	15mLx2	70mL
Reagent 5B	DAB Chromogen(20x)	1.5mL	2mL	3mL
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.
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. **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:

fume absorbing hood, etc.	Peroxidase and alkaline phosphatase blocking buffer
Heat source (microwave or hot plate) for antigen retrieval buffers.	100% ethanol
Thermometer	100% Xylene
Beaker	Hematoxylin

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Timer	Wash buffer: 0.01M PBS with 0.5% Tween20, pH7.4
coverslip	Wash buffer: 50mM Tris-HCl, 150mM NaCl, 0.05% Tween-20 pH7.6

Steps / Reagent	**Staining Procedure**	**Incubation Time**
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. Refer to antibody datasheet. 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 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-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30-60 min.
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 only with **1X TBS-T**, 3 times for 2 minutes each.	15min.
5. **Reagent 2A, 2B&2C** **Reagent 2A:** GBI-Permanent Red Substrate (RTU) **Reagent 2B:** GBI-Permanent Red Activator (5x **Reagent 2C:** GBI-Permanent Red Chromogen (100x) (**To get maximum sensitivity of AP polymer, Please repeat chromogen step)**	**Note**: Shake GBI-Permanent Red Activator before adding into GBI-Permanent Red Substrate. a. Add 200µL of **Reagent 2B** (Activator) into 1mL of **Reagent 2A** (Substrate) and mix well. Add 10µL of **Reagent 2C**(Chromogen) into the mixture and mix well. [**Note:** For fewer slides, add 100µL of **Reagent 2B** (Activator) into 500µL of **Reagent 2A** (Substrate) and mix well. Add 5µL of **Reagent 2C**(Chromogen) into the mixture and mix well.] b. Apply 2 drops (100µL) or enough volume of GBI-Permanent Red working solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development. **To increase AP signal aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the GBI-Permanent Red working solution to completely cover the tissue for additional 5 to 10min.** c. Rinse well with distilled water. d. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	10min.
6. **Reagent 3** DS-GM Blocker (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 3** (DS-GM Blocker) to cover each section. b. Incubate in moist chamber for 10 min. c. Blot off solution. Rinse with PBS/Tween-20 (0.05%) or **1xTBS-T** once for 5sec.	10min.
7**. Reagent 4** Mouse HRP Polymer (RTU)	a. Apply 1 to 2 drops (50-100µL) of **Reagent 4** (Mouse HRP 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
8. **Reagents 5A, 5B:** **5A:** DAB Substrate(RTU) **5B:** DAB Chromogen(20x)	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of **Reagent 5B** to 1 mL of **Reagent 5A**. Mix well. Protect from light and use within 7 hours at 4℃. b. Apply 2 drops or enough volume of DAB working	5min.

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	solution to completely cover tissue. Incubate for 5 min. c. Rinse thoroughly with distilled water.	
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) **To coverslip see protocol note 2**.	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

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. **GBI-Permanent Red** is insoluble in organic solvent and can be coverslipped as well. however the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.
   Note: Please wipe off extra water and air dry slides before dehydration and clear.
   a. 1x 80% Ethanol 20 seconds;
   b. 1x 95% Ethanol 20 seconds;
   c. 3x 100% Ethanol 20 seconds each;
   d. 1x 100% Xylene 20 seconds;
   e. Add 1 drop of xylene based mountant (Cat. No. O-Mount, E02-18) and coverslip. Press to push the air bubble out.

CAUTION: DO NOT dehydrate in xylene longer than 20 seconds! It will erase GBI-Permanent Red stain!

Precautious:
DAB may be carcinogenic. 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 DS207A 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) **Wash with 1xTBS-T only.**				
Step 5	**Reagent 2A, 2B & 2C** GBI Permanent Red requires mixing (10min)				
Step 6	**Reagent 3** DS-GM Blocker RTU (10min) Rinse with PBS then Go immediately to step 6				
Step 7	**Reagent4** Mouse HRP Polymer RTU (15min)				
Step 8	**Reagent 5A&5B** DAB requires mixing (5min)				
Step 9	Counter stain Hematoxylin User supplied				
Step 10	**Reagent 6** Simpo-Mount RTU				

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	Do not coverslip!				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Testing result:

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