Polink DS-MR-Hu A1 Kit for Immunohistochemistry Staining

DS201 Polymer HRP & AP Double Staining Kit Detects Mouse & Rabbit Primary Antibodies on Human Tissue with DAB (Brown) and GBI-Permanent Red (Red).

Storage: 2-8ºC

Catalog No.:
☐ DS201A-6 12mL* 120 slides**
☐ DS201A-18 36mL* 360 slides**
☐ DS201A-60 120mL* 1200 slides**
**Total volume of polymer Conjugates*
*** if use 100µl per slide*

Intended Use:
The **Polink DS-MR-Hu A1 Kit** is designed to use with user supplied mouse and rabbit antibodies to detect two distinct antigens on human tissue or cell samples. This kit has been tested in paraffin embedded tissues. However, this kit can be used to stain frozen specimen and/or freshly prepared monolayer cell smears.

Double staining is a common method used in immunohistochemistry to evaluate two distinct antigens in a single tissue[1, 2]. GBI Labs **Polink DS-MR-Hu A1 Kit** contains a HRP-Polymer anti-Mouse IgG, an AP-Polymer anti-Rabbit IgG, and two distinct chromogens. The DAB chromogen (brown color) is used with the HRP-Polymer anti-Mouse IgG and GBI-Permanent Red(red color) is used with the AP-Polymer anti-Rabbit IgG. Simplified steps offer a much faster protocol as the enzyme conjugates are applied to the specimen as a mixture. **Polink DS-MR-Hu A1 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-Mouse (RTU)	6mL	18mL	60mL
Reagent 2	AP Polymer anti-Rabbit (RTU)	6mL	18mL	60mL
Reagent 3A	DAB Substrate (RTU)	12mL	18mLx2	120mL
Reagent 3B	DAB Chromogen (20x)	1.5mL	2mL	6mL
Reagent 4A	GBI-Permanent Red Substrate (RTU)	15mL	18mLx2	120mL
Reagent 4B	GBI-Permanent Red Activator (5x)	3mL	7.2mL	12mLx2
Reagent 4C	GBI-Permanent Red Chromogen (100x)	150µL	360µL	1.2mL
Reagent 5	Simpo-Mount (RTU)	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 deparaffinized 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 with 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
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 at least twice.	10 min.
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 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	***Notes:*** Investigator needs to optimize dilution and incubation times prior to double staining. a. Apply 2 drops or enough volume of both Primary Antibody 1 and	30-60 min.

22310 20th Ave SE#100. Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

	Antibody 2 to cover the tissue completely. Mix well on the slide and Incubate in moist chamber for 30-60 min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	
5. **Reagent 1 and 2:** **Reagent 1 :** HRP Polymer anti-Mouse (RTU) **Reagent 2:** AP Polymer anti-Rabbit (RTU)	a. Apply 1drop (50µl) of **Reagent 1** (HRP Polymer anti-Mouse) and 1 drop of **Reagent 2** (AP Polymer anti-Rabbit) to cover each section, mix well on the slide. Or you may prepare secondary antibodies cocktail in advance: 50µl **Reagent 1** (HRP Polymer anti-Mouse) plus 50µl **Reagent 2** (AP Polymer anti-Rabbit) per slide. b. Incubate in moist chamber for 30 min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30 min.
6. **Reagents 3A, 3B:** **Reagent 3A:** DAB Substrate(RTU) **Reagent 3B:** DAB Chromogen(20x)	a. Add 1 drop of or 2 drops (for higher sensitivity and contrast) **Reagent 3B** to 1 mL of **Reagent 3A**. Mix well. Protect from light and use within 7 hours. b. Apply 2 drops or enough volume of DAB CHROMOGEN to completely cover tissue. Incubate for 5 min. c. Rinse thoroughly with distilled water. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	5 min.
7. **Reagent 4A, 4B, 4C** **Reagent 4A:** GBI-Permanent Red Substrate (RTU) **Reagent 4B:** GBI-Permanent Red Activator (5x) **Reagent 4C:** GBI-Permanent Red Chromogen (100x) (**To get maximum sensitivity of AP polymer, Please repeat chromogen step)**	**Note: Make fresh working solution and use immediately. Shake GBI-Permanent Red Activator well before adding into GBI-Permanent Red Substrate.** a. Add 200µL of **Reagent 4B** (Activator-shake well) into 1mL of **Reagent 4A** (Substrate buffer) and mix well. Add 10µL of **Reagent 4C** (Chromogen) into the mixture and mix well. [**Note:** For fewer slides, Add 100µL of **Reagent 4B** (Activator) into 500µL of **Reagent 4A** (Substrate buffer) and mix well. Add 5µL of **Reagent 4C** (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, make fresh working solution again, tap off previous chromogen, apply 2-3 drops (100µL) immediately and incubate additional 10min.** c. Rinse well with distilled water.	10min OR (10 min + 10 min)
8. 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	10-15sec
9. **Reagent 5:** Simpo-Mount(RTU)	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. 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.	

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 coversliped 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. Use fresh ethanol and xylene.
   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

22310 20th Ave SE#100. Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

Work Sheet for DS201A 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

DS201A Protocol is suitable when both mouse and rabbit primary antibodies need or do not need pre-treatment step.

Protocol Step	**DS201A Protocol Reagent / Time**	**Experiment 1 Date:**	**Experiment 2 Date:**	**Experiment 3 Date:**	**Experiment 4 Date:**
Step 1	Peroxidase or Alkaline Phosphatase Block User supplied				
Step 2 (Optional)	HIER if needed User supplied (up to 60 min)				
Step 3	Mouse 1°Ab & Rabbit 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1& Reagent 2** HRP-Polymer anti-Mouse IgG and AP-Polymer anti-Rabbit IgG require mixing (30min)				
Step 5	**Reagent 3A & Reagent 3B** DAB Requires mixing! (5 min.)				
Step 6	**Reagent 4A, Reagent 4B& Reagent 4C** **Note: Make fresh working solution and use immediately. Shake Reagent 4B well before adding into Reagent 4A.** GBI-Permanent Red Requires mixing! (10 min) **To increase sensitivity, please repeat this step.**				
Step 7	Counter stain(10-15sec) User supplied				
Step 8	**Reagent 5** Simpo Mount (RTU)				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

The result:

22310 20th Ave SE#100. Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com