

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: 2-8°C

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:

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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
 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	<i>Notes:</i> 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.

	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:	a. Apply 1drop (50µl) of Reagent 1 (HRP Polymer anti-Mouse) and 1	
Reagent 1 : HRP Polymer anti-Mouse	drop of Reagent 2 (AP Polymer anti-Rabbit) to cover each section, mix	
(RTU)	well on the slide. Or you may prepare secondary antibodies cocktail in	
Reagent 2: AP Polymer anti-Rabbit	advance: 50µl Reagent 1 (HRP Polymer anti-Mouse) plus 50µl Reagent 2	20
(RTU)	(AP Polymer anti-Rabbit) per slide.	30 min.
	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.	
6. Reagents 3A, 3B:	a. Add 1 drop of or 2 drops (for higher sensitivity and contrast) Reagent	
Reagent 3A:	3B to 1 mL of Reagent 3A . Mix well. Protect from light and use within	
DAB Substrate(RTU)	7 hours.	
Reagent 3B:	b. Apply 2 drops or enough volume of DAB CHROMOGEN to	5 min.
DAB Chromogen(20x)	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.	
7. Reagent 4A, 4B, 4C	Note: Make fresh working solution and use immediately. Shake GBI-	
	Permanent Red Activator well before adding into GBI-Permanent	
Reagent 4A:	Red Substrate.	
GBI-Permanent Red Substrate (RTU)	a. Add 200µL of Reagent 4B (Activator-shake well) into 1mL of Reagent	
Reagent 4B:	4A (Substrate buffer) and mix well. Add 10µL of Reagent 4C	
GBI-Permanent Red Activator (5x)	(Chromogen) into the mixture and mix well.	
Reagent 4C:	[Note: For fewer slides, Add 100µL of Reagent 4B (Activator) into	10min
GBI-Permanent Red Chromogen (100x)	500µL of Reagent 4A (Substrate buffer) and mix well. Add 5µL of	OR
(To get maximum sensitivity of AP	Reagent 4C (Chromogen) into the mixture and mix well.]	$(10 \min + 10 \min)$
polymer, Please repeat chromogen	b. Apply 2 drops (100µL) or enough volume of GBI-Permanent Red	· · · · · ·
step)	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.	
8. HEMATOXYLIN	a. Counterstain with 2 drops (100µl) or enough volume of hematoxylin to	
Not provided	completely cover tissue. Incubate for 10-15 seconds.	10.15
*	b. Rinse thoroughly with tap water for 2-3 min.	10-15sec
	c. Put slides in PBS until show blue color (about $\frac{1}{2}$ - 1 min.)	
	d. Rinse well in distilled water	
9. Reagent 5:	a. Apply 2 drops (100µL) or enough volume of Reagent 5 Simpo-Mount	
Simpo-Mount(RTU)	to cover tissue when tissue is wet. Rotate the slides to allow Simpo-	
1	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.	
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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. **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. <u>De Pasquale A, Paterlini P, Quaglino D</u>. *Immunochemical demonstration of different antigens in single cells in paraffin-embedded histological sections*. <u>Clin Lab Haematol.</u> 1982;4(3):267-72.

2. Polak J. M and Van Noorden S. Introduction to Immnocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997

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 " $\sqrt{}$ "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: