Polink DS-RRt-Hu/Ms A Kit

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

Storage: 2-8ºC

Catalog No.: ☐ DS211A-6 12mL* 120 slides**
☐ DS211A-18 36mL* 360 slides**
☐ DS211A-60 120mL* 1200 slides**
**Total volume of polymer Conjugates*
***If using 100µL per slide*

Intended Use:
The **Polink DS-RRt-Hu/Ms A Kit** is designed for use with user supplied rabbit and rat primary antibodies to detect two distinct antigens on human and mouse tissue or cell samples. This kit has been tested on paraffin embedded tissue. 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 immunohistostaining, allowing for the detection of two distinct antigens in a single tissue. Polink DS-RRt-Hu/Ms A Kit from GBI labs supplies the user with two polymer enzyme conjugates: HRP polymer anti-rat IgG (minimal cross reaction to mouse) and AP polymer anti-rabbit IgG with two distinct substrates/chromogens, DAB and GBI-Permanent Red. DAB chromogen reacts with the HRP polymer anti-Rat conjugate to produce a brown color. GBI-Permanent Red reacts with AP polymer anti-Rabbit conjugate to produce the subsequent red color. Polink DS-RRt-Hu/Ms A Kit is a non-biotin system avoiding the extra steps involved in blocking non-specific binding due to endogenous biotin.

Kit Components:

Component No.	Content	12mL Kit	36mL Kit	120mL Kit
Reagent 1	Rabbit AP Polymer (RTU)	6mL	18mL	60mL
Reagent 2	Rat-NM HRP Polymer (RTU)	6mL	18mL	60mL
Reagent 3A	DAB Substrate (RTU)	12mL	30mL	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)	15mL	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 needs to be adhered to the slide tightly to avoid falling off.
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 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. Proceed with IHC staining: **DO NOT** let specimen or tissue dry from this point on.
7. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting results.
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 pH 7.6. GBI sells 10xTBS-T for your convenience (B11xx).

Steps / Reagent	Staining Procedure	Incubation Time
1. Peroxidase and Alkaline Phosphatase Blocking Reagent: Not provided	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 8 above)**; 3 times for 2 minutes each.	Up to 1 hour
3. Primary Antibody Mix: **one Rat and one Rabbit antibody:** Supplied by user	**Note:** Investigator needs to optimize dilution prior to double staining. a. Apply 2 drops (100 µL) or enough volume of rat and rabbit primary antibody mixture to cover the tissue completely. Incubate in moist chamber for 30-60 min. Recommend 30 min 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

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4. **Reagent 1:** Rabbit AP Polymer (RTU)	a. Apply 1 to 2 drops of the **Reagent 1** (Rabbit AP Polymer) to cover each section. b. Incubate in moist chamber for 15-30 min. c. Wash with **1X TBS-T only**; 3 times for 2 minutes each. **Note:** longer incubation may increase background.	15-30 min
5. **Reagent 2:** Rat-NM HRP Polymer (RTU)	a. Apply 1 to 2 drops of **Reagent 2** (Rat-NM HRP Polymer) to cover each section. b. Incubate in moist chamber for 15-30 min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	15-30 min
6. **Reagents 3A, 3B:** **Reagent 3A:** DAB Substrate (RTU) **Reagent 3B:** DAB Chromogen (20x)	**Note:** Make enough DAB mix by adding 1 drop of **Reagent 3B** (DAB Chromogen) in 1mL of **Reagent 3A** (DAB Substrate). Mix well. Use within 7 hours. a. Apply 1 to 2 drops (50-100µL) of your DAB working solution to cover the tissue completely. b. Incubate for 5 min. c. Rinse slides in multiple changes of distilled water 3 times, 2 min each time or under running tap water for 1 min. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	5 min
7. **Reagents 4A, 4B, 4C:** **Reagent 4A:** GBI-Permanent Red Substrate (RTU) **Reagent 4B:** GBI-Permanent Red Activator (5x **Reagent 4C:** GBI-Permanent Red Chromogen (100x)	Note: Shake GBI-Permanent Red Activator before adding into GBI- Permanent Red Substrate. a. Add 200µL of **Reagent 4B** (Activator) into 1mL of **Reagent 4A** (Substrate buffer) and mix well. Add 12µL 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 6µL 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. **(To get maximum sensitivity of AP polymer, repeat chromogen step)**	10 min OR (10min+10min)
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 30 - 60 sec). d. Rinse well in distilled water.	
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 to spread evenly.	

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!

Precautions:

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 Immunocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997

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Work Sheet for DS211A Kit

We designed this work sheet to help you 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.
To ensure 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 de-paraffinization
- Refer to insert for details of each step

DS211A Protocol is suitable when both Rabbit and rat primary antibodies need or do not need pre-treatment step.

Step/ Protocol	**Protocol DS211A**	**Experiment 1 Date:**	**Experiment 2 Date:**	**Experiment 3 Date:**	**Experiment 4 Date:**
Step 1	Peroxidase or Alkaline Phosphatase Block E36 is recommended. supplied				
Step 2	HIER if needed				
Step 3	Rb 1°Ab & Rat 1°Ab mix (30-60 min.)				
Step 4	**Reagent 1:** Rabbit AP Polymer (15-30 min)				
Step 5	**Reagent 2:** Rat-NM HRP Polymer (15-30 min)				
Step 6	**Reagent 3A &Reagent 3B:** DAB requires mixing (5min)				
Step 7	**Reagent 4A, Reagent 4B, & Reagent 4C:** GBI-Permanent Red requires mixing (10min)				
Step 8	Counter stain: User supplied				
Step 9	**Reagent 5:** Simpo Mount (RTU)				
Result	Stain pattern on controls is correct: Fill in Yes or NO				

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