Polink DS-GR-Hu/Ms A Kit for Immunohistochemistry Staining

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

Storage: 2-8ºC

Catalog No.:
- ☐ DS205A-6 12mL* 120 slides**
- ☐ DS205A -18 36mL* 360 slides**
- ☐ DS205A -60 120mL* 1200 slides**

Total volume of polymer Conjugates

***If use 100µL per slide*

Intended Use:

Polink DS-GR-Hu/Ms A Kit is designed to use with user supplied goat and rabbit primary antibodies, to detect two distinct antigens on human and mouse 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 commonly methods used in immunohistostaining for revealing two distinct antigens in a single tissue[1, 2]. **Polink DS-GR-Hu/Ms A Kit** from GBI Labs (Golden Bridge International) supplies two polymer enzyme conjugates: HRP Polymer anti-Goat IgG and AP Polymer anti-Rabbit IgG with two substrates/chromogens, DAB (Brown) and GBI-Permanent Red (Red). Simplified steps offer a convenient protocol as the enzyme conjugates are applied to the specimen simultaneously. If only the anti-goat antigen is present, HRP polymer will result with DAB(brown) chromogen will be present and if only the anti-rabbit antigen is present, AP polymer will react only with GBI-Permanent Red(red) chromogen. When both rabbit and goat antigen is present both DAB and GBI-Permanent Red chromogen will be present. **Polink DS-GR-Hu/Ms A Kit** is a non-biotin system, avoiding blocking steps for endogenous biotin non-specific binding.

Kit Components:

Component No.	Content	6mL Kit	36mL Kit	120mL Kit
Reagent 1	Goat HRP Polymer (RTU)	6mL	18mL	60mL
Reagent 2	Rabbit AP Polymer (RTU)	6mL	18mL	60mL
Reagent 3A	DAB Substrate (RTU)	15mL	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)	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 deparffinized with xylene and rehydrated with a graded series of alcohols 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. DO NOT let specimen or tissue dry during protocol.
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. 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.	10-20min
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.	
3. Primary Antibody Mix: **one Goat**	**Note:** Investigator needs to optimize dilution prior to double staining.	30-60min

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and one Rabbit antibody Supplied by user	a. Apply 2drops (100µL) or enough volume of goat and rabbit primary antibodies mixture to cover the tissue completely. Incubate in moist chamber for 30-60min. 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.	
4.Mix **Reagent 1:** Goat HRP Polymer (RTU) with **Reagent 2** Rabbit AP Polymer (RTU)	**Note:** Only make enough mixture for the experiment performed. Mixture is not stable for long term storage. Make sufficient polymer mixture by adding **Reagent 1** Goat HRP Polymer and **Reagent 2** Rabbit AP Polymer at 1:1 ratio, mix well. a. Apply 2 drops (100µL) or enough volume of the mixture to cover each section. b. Incubate in moist chamber for 15min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	15min
5. **Reagent 3A and 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. Store at 4°C protecting from light and use within 7 hours. a. Apply 1 to 2 drops (50-100µL) of DAB working solution to cover the tissue completely. b. Incubate for 5min. c. Rinse slides with distilled water 2min 3 times, or running tap water for 1min. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each	5min
6. **Reagent 4A, 4B, 4C** **Reagent 4** 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**: 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 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 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.	10min
7. Counterstain (**Optional**) Not provided	a. Counterstain with 2 drops (100µL) or enough volume of counterstain solution to completely cover tissue. Incubate for 10-15sec. b. Rinse thoroughly with tap water for 2-3min. c. Rinse well in distilled water.	10-15sec
8. **Reagent 5:** Simpo-Mount (RTU)	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.	

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.
   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.

Precautious:

Please wear gloves, eye protection and take other necessary precautions. If any of the reagent come in contact with skin wash area completely with plenty of water and soap. If irritation develops seek medical attention.

Remarks:

This kit is for research use only.

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Work Sheet for DS205A Kit

We designed this work sheet to help you keep 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.

- Used for tester to check "√ "each step during the experiment
- Steps follow after de-paraffinization
- Refer to insert for details of each step

DS205A Protocol is suitable when both goat and rabbit primary antibodies need or do not need pre-treatment step.

Step/ Protocol	Protocol of DS205A	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase& alkaline phosphatase Block User supplied				
Step 2	HIER if needed User supplied				
Step 3	Gt 1°Ab & Rb 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** Goat HRP Polymer (RTU)& Rabbit AP Polymer (RTU) require mixing 30min				
Step 5	**Reagent 3A & Reagent 3B** DAB requires mixing 5min				
Step 6	**Reagent 4A, 4B & 4C** GBI-Permanent Red Requires mixing! 10min				
Step 7	Counter stain 10-15sec Hematoxylin User supplied				
Step 8	**Reagent 5** Simpo-Mount (RTU)				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Testing result:

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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