Polink DS-GR-Hu/Ms D 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 Fast Red (Red)

Storage: 2-8°C

Catalog No.:
- ☐ DS205D-6 12mL* 120 slides**
- ☐ DS205D-18 36mL* 360 slides**
- ☐ DS205D -60 120mL* 1200 slides**

**Total volume of polymer Conjugates*

***If use 100µL per slide*

Intended Use:

Polink DS-GR-Hu/Ms D 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 D 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 Fast 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 Fast Red(red) chromogen. When both rabbit and goat antigen is present both DAB and Fast Red 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	Fast Red Chromogen	6 tablets	18 tablets	60 tablets
Reagent 4B	Fast Red Substrate(RTU)	5ml x 6	5ml x 18	5ml x 60
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 (Min.)
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 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 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 and one Rabbit**	**Note:** Investigator needs to optimize dilution prior to double staining. a. Apply 2drops (100µL) or enough volume of goat and rabbit primary	30-60min

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antibody Supplied by user	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 30min. c. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30min
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:** Fast Red Chromogen It takes about **30 minutes** to dissolve the tablet in the substrate buffer. Allow enough time to prepare.	a. Dissolve 1 tablet of **Reagent 4A** (Fast Red Chromogen) in 5ml **Reagent 4B** (Fast Red Substrate), vortex until the tablet dissolved completely. Use within 1 hour. b. Apply 2 drops (100µL) or enough volume of Fast Red working solution to completely cover the tissue. Incubate for 10-20 min, observe appropriate color development c. Rinse well with distilled water. (**Fast Red is alcohol soluble; do not dehydrate.**)	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.	
8. **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. 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. **To coverslip see protocol note 2**.	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. Simpo-Mount is an aqueous-based mounting media for immunohistochemistry. It is used as the permanent mounting media for alcohol soluble chromogens such as Fast Red, AEC, and BCIP. Simpo-Mount does not use a coverslip. However, if you need to coverslip your tissue, after Simpo-Mount has dried, dip the slide in xylene (1 to 2 seconds), apply an organic mounting solution (such as O-Mount, Cat# E02-18), and place cover glass on the slide. Store slides after they have dried completely.

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.

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 DS205D 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

Step/ Protocol	Protocol of DS205D	Experiment 1 Date:	Experiment 2 Date:	Experiment 3 Date:	Experiment 4 Date:
Step 1	Peroxidase& alkaline phosphatase Block				
Step 2	HIER if needed				
Step 3	Gt 1°Ab & Rb 1°Ab mixture (30-60 min.)				
Step 4	**Reagent 1 & Reagent 2** Goat AP Polymer (RTU)& Rabbit HRP Polymer (RTU) require mixing 30min				
Step 5	**Reagent 3A & Reagent 3B** DAB requires mixing 5min				
Step 6	**Reagent 4A &Reagent 4B** Fast Red Requires mixing! 10min				
Step 7	Counter stain Hematoxylin User supplied				
Step 8	**Reagent 5** Simpo-Mount (RTU) Do not coverslip!				
Result	Stain pattern on controls are correct: Fill in Yes or NO				

Testing result:

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