Polink DS-MR-Hu D1 Kit for Double Staining (DAB /Fast Red) Kit

(Simultaneous polymer double staining kit for mouse and rabbit antibody
With DAB and Fast Red chromogen)

Fewer steps and better result than sequential procedure

Storage: 2-8ºC

Catalog No.: ☐ DS201D-6/(D20-6F) 12ml* 120 slides**
☐ DS201D-18 36ml* 360 slides**
☐ DS201D-60 120ml* 1200slides**

**Total volume of polymer Conjugates*
*** if use 100µl per slide*

Intended Use:
The **Polink DS-MR-Hu D1 Kit** is designed to use with user supplied mouse antibody and rabbit antibody to detect two distinct antigens on human 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 common methods used in immunohistostaining that allow revealing two distinct antigens in a single tissue1, 2. **Polink DS-MR-Hu D1** Kit from Golden Bridge International supplies two polymer enzyme conjugates: HRP Polymer anti-Mouse IgG and AP Polymer anti-Rabbit IgG with two distinct substrates/chromogens, DAB (brown color, use with HRP Polymer anti-Mouse IgG) and Fast Red (red color, use with AP Polymer anti-Rabbit IgG). User may apply the two enzyme conjugates onto the specimen at the same time and mix them on the slide. Simplified steps offer user much faster and quicker protocol than a sequential procedure. **Polink DS-MR-Hu D1 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 IgG(RTU)	6 ml	18ml	60ml
Reagent 2	AP Polymer anti-Rabbit IgG(RTU)	6 ml	18ml	60ml
Reagent 3A	DAB substrate buffer (RTU)	12 ml	36ml	120ml
Reagent 3B	DAB chromogen (20x)	1.5 ml	2ml	6ml
Reagent 4A	Fast Red chromogen tablets	6 tablets	18 tablets	60 tablets
Reagent 4B	Fast Red substrate buffer (RTU)	5ml x 6	5ml x 18	5ml x 60
Reagent 5	Simpo-Mount solution (RTU)	12 ml	36ml	120 ml

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 deparffinized 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. It takes about 30 minutes to dissolve Fast Red tablet into the substrate buffer. Make sure to start preparing Fast Red solution near the end of the secondary antibody incubation.
7. Proceed IHC staining: DO NOT let specimen or tissue dry from this point on.
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 distilled water.	10 min

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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. 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 1and 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.	30-60 min
5. **Reagent 1 and 2: Reagent 1:** HRP Polymer anti-Mouse IgG (RTU) **Reagent 2:** AP Polymer anti-Rabbit IgG (RTU)	a. Apply 1drop (50µl) of **Reagent 1** HRP Polymer anti-Mouse IgG and 1 drop of **Reagent 2** AP Polymer anti-Rabbit IgG 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 IgG plus 50µl **Reagent 2** AP Polymer anti-Rabbit IgG per slide. b. Incubate in moist chamber for 30 min. b. 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:** **3A:** DAB substrate buffer (RTU) **3B:** DAB Chromogen(20X)	a. Add 1 drop or **2 drops** (for higher sensitivity and contrast) of Reagent **3B** to 1 ml Reagent **3A**. Mix well. Protect from light and use within 5 hours. b. Apply 2 drops or enough volume of DAB CHROMOGEN to completely cover tissue. Incubate for 3-10 min. c. Rinse well with distilled water. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	3-10 min
7. **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 **Fast Red tablet** in 5ml **Fast Red substrate buffer**, vortex until the tablet dissolved completely. Use within 1 hour. b. Apply 2 drops (100µl) or enough volume of Fast -Red 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.**)	10-20 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	
9. **Reagent 5:** Simpo-Mount	a. Apply 2 drops (100µl) or enough volume of **Reagent 5** 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.	30 min. 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 effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.
2. Simpo-Mount is water-based mounting medium for immunohistology. It may be used as the permanent mounting media. It does not need coverslip. However, if you need to coverslip, after Simpo-Mount dried, dip the slide in xylene and take out immediately. Apply O-Mount (organic mounting solution, Catalog No. E02-15) on the tissue and place cover glass on the slide. Store it after dry completely.

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

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