Polink DS-MR-Hu D2 Kit for Immunohistochemistry Staining

Polymer-HRP&AP double staining kit to distinct a rabbit and a mouse primary antibody on Human tissue with DAB (Brown) and Fast Red (Red)

Storage: 4-8ºC

Catalog No.: ☐ DS202D-6/(D32-6F) 12mL* 120 slides**
☐ DS202D-18 36mL* 360 slides**
☐ DS202D-60 120mL* 1200 slides**
**Total volume of polymer Conjugates*
*** if use 100µL per slide*

Intended Use:
The **Polink DS-MR-Hu D2 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 tissue[1, 2]. **Polink DS-MR-Hu D2 Kit** from Golden Bridge International supplies two polymer enzyme conjugates: HRP-Polymer anti-Rabbit IgG and AP-Polymer anti-Mouse IgG with two distinct substrates/chromogens, DAB (brown color, use with HRP-Polymer anti-Rabbit IgG) and Fast Red (red color, use with AP-Polymer anti-Mouse 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 D2 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-Rabbit(RTU)	6mL	18mL	60mL
Reagent 2	AP- Polymer anti-Mouse(RTU)	6mL	18mL	60mL
Reagent 3A	DAB Substrate Buffer (RTU)	12mL	18mLx2	120mL
Reagent 3B	DAB Chromogen(20x)	1.5mL	2mL	6mL
Reagent 4A	Fast Red Tablet	6tablets	18tablets	60tablets
Reagent 4B	Fast Red Substrate(RTU)	5mLx6	5mLx18	5mLx60
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 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. Proceed 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 (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.	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 7 above)**; 3 times for 2 minutes each.	
3. Preblock	For paraffin section, Improved formula saves the need for a preblock step.	

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(optional)	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	***Note:*** 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.	30-60min
5. **Reagent 1 and 2: Reagent 1:** HRP-Polymer anti Rabbit (RTU) **Reagent 2:** AP- Polymer anti Mouse(RTU)	***Note*:** Make sufficient polymer mixture by adding **Reagent 1** HRP-Polymer anti-Rabbit and **Reagent 2** AP-Polymer anti-Mouse at 1:1 ratio, mix well. Do Not Mix More than you need for the experiment because the polymer mixture may not be as stable as non-mixed polymer. a. Apply 1 to 2 drops (50-100µL) of the mixture to cover the tissue completely. 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.	30min
6. **Reagents 3A, 3B:** **Reagents 3A:** DAB Substrate Buffer (RTU) **Reagents 3B:** DAB Chromogen(20x)	a. Add 1 drop of or 2 drops (for higher sensitivity and contrast) **Reagent 3B** to 1 mL of **Reagent 3A**. Mix well. Protect from light and use within 7 hours. b. Apply 2 drops or enough volume of DAB CHROMOGEN to completely cover tissue. Incubate for 3-10min. c. Rinse thoroughly with distilled water. d. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	3-10min
7. **Reagent 4A, 4B:** **Reagent 4A:** Fast Red Tablet **Reagent 4B:** Fast Red Substrate(RTU)	***Notes:*** 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 Tablet in 5mL **Reagent 4B** 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 10-20min, observe appropriate color development. c. Rinse well with distilled water. (**Fast Red is alcohol soluble; do not dehydrate.**)	10-20min
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 2-3min. c. Put slides in PBS until show blue color (about ½ - 1min.) 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 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.	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 effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.
2. Simpo-Mount is a water-based mounting medium for immunohistochemistry. It is used as the permanent mounting media for alcohol soluble chromogens such as AP-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:

DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

Remarks:

For research use only.

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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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