Polink-2 AP Broad Detection System

(Polymer-Alkaline Phosphatase Detection Kit for Mouse and Rabbit Primary Antibodies)

Second Generation Biotin Free Polymer Detection System

Storage: 4-8ºC

Catalog No. ☐ D24-110 110 mL (bulk, w/o chromogen)
☐ D24-18 18 mL (now with chromogen)
☒ D24-6 6 mL (now with chromogen)

Intended Use:

Polink-2 Detection kit is an alkaline phosphatase (AP) polymer detection system that is used for detecting mouse and rabbit primary antibodies bound to tissue sections. Polink-2 kit is the second generation polymer detection system that uses polymer helper and polymeric AP-linked antibody conjugates to get consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies, especially on some nuclear-stained antibodies. This technology provides excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving.

Kit components:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	AP Polymer Helper (RTU)	6mL	18mL	110mL
Reagent 2	AP Polymer anti-Mouse/Rabbit(RTU)	6mL	18mL	110mL
Reagent 3A	GBI-Permanent Red Substrate (RTU)	7mL	18mL	NA
Reagent 3B	GBI-Permanent Red Activator (5x)	1.4mL	3.6mL	NA
Reagent 3C	GBI-Permanent Red Chromogen (100x)	70µL	180µL	NA

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 deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made into as thin of a monolayer as possible to obtain satisfactory results.
5. Investigator needs to optimize dilution and incubation times for primary antibodies.
6. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
7. Staining steps: 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 inhibitor the activity of the alkaline phosphatase. **Note: 1X TBS-T** =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6

Reagent	Staining Procedure	Incubation Time
1. Alkaline Phosphatase Blocking Reagent (Not provided)	a. Incubate slides in alkaline phosphatase blocking reagent. Note: E36xx is recommended. b. Rinse the slide using distilled water.	Refer to datasheet
2. HIER PRETREATMENT:	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. b. Wash with PBS-T 3 times for 2 minutes each time. See #8 above for use of TBS-T.	
3. PRIMARY ANTIBODY Supplied by user	a. Apply 2 drops (100µL) or enough volume of PRIMARY ANTIBODY to cover the tissue section completely. Incubate in moist chamber for 30-60 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 minutes each time for 3 times.	30-60 min.
4. **Reagent 1:** AP Polymer Helper (RTU)	a. Apply 2 drops (100µL) or enough volume of AP **Reagent 1** Polymer Helper to cover each section. Incubate in moist chamber for 15- 20 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 minutes each time for 3 times.	15-20 min.
5. **Reagent 2** AP Polymer anti-Mouse/Rabbit (RTU)	a. Apply 2 drops (100µL) or enough volume of AP Polymer anti-Mouse/Rabbit to cover each section. Incubate in moist chamber for 15-20 min. b. Rinse with PBS containing 0.05% Tween-20 for 2 minutes each time for 3 times. c. Rinse with tap water(or TBS-T which may increase sensitivity.).	15-20 min.
6. **Reagent 3A, 3B, 3C** **Reagent 3A:** GBI-Permanent Red Substrate	a. Add 200µL of **Reagent 3B** (Activator) into 1mL of **Reagent 3A** (Substrate buffer) and mix well. Add 10µL of **Reagent 3C**(Chromogen) into the mixture and mix well. [**Note:** For fewer slides, Add 100µL of **Reagent 3B** (Activator) into 500µL of **Reagent 3A** (Substrate buffer) and mix well. Add 5µL of **Reagent 3C**(Chromogen)	10 min

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(RTU) **Reagent 3B:** GBI-Permanent Red Activator (5x) **Reagent 3C:** GBI-Permanent Red Chromogen (100x)	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. c. Rinse well with distilled water.	
7. HEMATOXYLIN Supplied by user	a. Counterstain with Hematoxylin for 20-30 seconds. b. Rinse slides under tap water for 1-2 minutes b. Put slides in PBS until show blue color (about 60-90 seconds) c. Rinse well in distill or tap water.	
8. SIMPO-MOUNT (Cat#E03-18 or E03-100) Not included	a. Apply 2 drops or enough volume of Simpo-Mount on the tissue. Must apply Simpo-Mount when tissue is wet. b. Rotate the slides to allow Simpo- Mount spread evenly to cover the tissue section, DO NOT cover slip on top of the Simpo-Mount. c. Place slides in an oven at 40-50°C for at least 30 minutes or leave it at room temperature until slides are thoroughly dried. Slow dry at room temperature will help to eliminate the air bubbles. Hardened Simpo-Mount forms an impervious permanent mount to organic solvents.	

Protocol Notes:

1. The fixation, tissue slide thickness, 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. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.
5. 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.
   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!

Related Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
Polink-2 HRP Broad Bulk kit for DAB (without DAB)	D22-110 D22-60	110mL 60mL		Fast Red Kit	C03-6	12 Tab + 60mL
Polink-2 HRP Broad DAB kit	D22-18 / D22-6	18mL/ 6mL		AP-Red+ Kit (40x)	C04-8	8mL
Polink-2 HRP Broad Bulk kit for AEC (without AEC)	D23-110 D23-60	110mL 60mL		BCIP/NBT Kit	C05-100 C05-18	100mL 18mL
Polink-2 HRP Broad AEC kit	D23-18 / D23-6	18mL/ 6mL		Simpo-Mount	E03-100/ E03-18	100mL/ 18mL
AEC kit (20x)	C01-12	12mL		DAB+ Kit (20x)	C09-12	12mL +240mL

Precautious:
Please wear gloves and take other necessary precautions.

Remarks:
For research use only.

References:

1. Bisgaard K, Pluzed KP. *Use of polymer conjugates in immunohitochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates.* Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungry, October 20-25, 1996.

2. Shi ZR. Itzkowitz SH, Kim YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcimoma tissues. J Hitochem Cytochem 36:317-322,

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