Polink-2 AP Broad-3 Detection Kit for Broad Spectrum

(Polymer-AP detection for mouse, rabbit, and goat primary antibodies using a biotin-free system)

Second Generation of Polymer Detection System

Storage: 4-8ºC

Catalog No.

☐	D27-110	110mL (good for 1100 slides)
☐	D27-18	18mL (good for 180 slides)
☐	D27-6	6mL (good for 60 slides)

Intended Use:

Polink-2 AP Broad-3 Detection Kit is an AP polymer based detection system that can be used to detect mouse, rabbit, or goat primary antibodies for immunohistochemical screens of human tissues. Polink-2 kits are a second generation polymer detection system that uses polymer helper and polymeric AP-linked antibody conjugates to get consistent immunostaining outcomes on archival tissues and to increase sensitivity on difficult-to-work antibodies, whether the antigen is cytoplasmic, membranous, or nuclear. 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. This kit has been tested on paraffin embedded tissue only; however it may be used on frozen or freshly prepared monolayer cell smears.

Kit components:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	Polymer Helper(RTU)	6mL	18mL	110mL
Reagent 2	Polymer AP anti-Mouse/Rabbit/Goat IgG(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, the 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 as much 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 as this will create uneven staining or background.
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. Supplied by user	a. Incubate slides in alkaline phosphatase blocking reagent. Note: E36xx is recommended.	10
	b. Rinse the slide using distilled water.	
2. HIER Pretreatment:	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. Please check the data sheet of primary antibody	
	b. Rinse with PBS with 0.05% Tween-20 for 2 min, 3 times. See #8 above for use of TBS-T.	
4. 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.	30-60
	b. Rinse with PBS with 0.05% Tween-20 for 2 min, 3 times.	
5. **Reagent 1** AP Polymer Helper (RTU)	a. Apply 2 drops(100µL) or enough volume of **Reagent 1** AP Polymer Helper to cover each section. Incubate in moist chamber for 15-20 min.	15-20

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	b. Wash with PBS with 0.05% Tween-20 for 2 min, 3 times.	
6. **Reagent 2** Polymer AP anti-Mouse/Rabbit/Goat (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 2** Polymer AP anti-Mouse/Rabbit/Goat to cover each section. Incubate in moist chamber for 15-20 min.	15-20
	b. Wash with PBS with 0.05% Tween-20 for 2 min, 3 times.	
7. **Reagent 3A, 3B, 3C** **Reagent 3A:** GBI-Permanent Red Substrate (RTU) **Reagent 3B:** GBI-Permanent Red Activator (5x) **Reagent 3C:** GBI-Permanent Red Chromogen (100x)	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) into the mixture and mix well.]	10
	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.	
8. HEMATOXYLIN Supplied by user	a. Counterstain with 2 drops (100µL) or enough volume of Hematoxylin to cover tissue completely and wait about 20-60 seconds.	20 seconds
	b. Rinse well with running tap water for 1-2 minutes.	
	c. Put slides in PBS until show blue color (about 30-60 seconds).	
	d. Rinse well in distilled or tap water.	
9. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. GB-Mount: Cat. No. E01-18 (18mL), for alcohol soluble substrates (AEC, GBI Permanent Red, and AP-blue) 2. O-Mount: Cat. No. E02-18 (18mL), for DAB 3. Simpo-Mount: Cat.No. E03-18 (18mL), or E03-100 (100mL), universal permanent mounting medium. Can be used with or without cover slip	Refer to insert

Protocol Notes:

1. The fixation, tissue slide thickness, 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. Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.
3. 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 AP Broad Bulk Kit (without chromogen)	D24-110	110mL		Fast Red Kit	C03-6	12 Tab + 60mL
Polink-2 AP Broad Kit (without chromogen)	D24-18 / D24-6	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
Polink-2 AP Broad-3 (Ms. Rb. Gt) Bulk Kit	D27-110	110mL		GBI-Permanent Red Kit	C13-120	120mL
Polink-2 AP Broad-3 (Ms. Rb. Gt) Bulk Kit	D27-18 / D27-6	18mL / 6mL		GBI-Permanent Red Kit	C13-18	18mL
AEC Kit (20x)	C01-12	12mL		DAB+ Kit (20x)	C09-12	12mL +240mL

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

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Remarks:
For research use only.

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