

**Polink-2 HRP DAB Broad-2 Detection System for Broad Spectrum**  
 (Polymer-HRP detection system, biotin-free, anti-mouse/rabbit primary antibodies)  
**Second Generation of Polymer Detection System**

Storage: 4-8°C
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- Catalog No.  D22-6 6mL (with DAB good for 60 slides)  
 D22-18 18mL (with DAB, good for 180 slides)  
 D22-60D 60mL (w/chromogen, good for 600)  
 D22-110 110mL (with DAB, good for 180 slides)  
 D22-1L 1000mL (good for 10,000 slides)

**Intended Use:**

Polink-2 HRP DAB Broad-2 Detection Kit is a HRP polymer based detection system that can be used to detect mouse or rabbit primary antibodies for immunohistochemical screens of human tissues. Polink-2 kits are a second generation polymer detection system that uses polymer helper and polymeric HRP-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	60mL Kit	110mL Kit
<b>Reagent 1</b>	Polymer Helper (RTU)	6mL	18mL	60mL	110mL
<b>Reagent 2</b>	Polymer HRP anti-Mouse/Rabbit IgG (RTU)	6mL	18mL	60mL	110mL
<b>Reagent 3A</b>	DAB Substrate (RTU)	12mL	15mLx2	NA	NA
<b>Reagent 3B</b>	DAB Chromogen (20x)	1.5mL	2mL	NA	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 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.
8. Serum blocking before primary antibody incubation for GBI's Polink-1, Polink-2, and Polink-2Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent	Staining Procedure	Incubation Time (Min.)
1. PEROXIDASE BLOCKING REAGENT. Supplied by user	a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H <sub>2</sub> O <sub>2</sub> solution) for 10 minutes.	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. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
3. PRE-BLOCK (Optional) Not provided	a. Add 2 drops (100µL) or enough volume of 10% Normal Goat Serum (E07) to completely cover the tissue section, Incubate for 10 min.	10

	b. Drain or blot off solution. DO NOT RINSE.	
	c. See note 8 in Recommended Protocol.	
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. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
5. <b>Reagent 1</b> Polymer Helper (RTU)	a. Apply 2 drops (100µL) or enough volume of <b>Reagent 1</b> Polymer Helper to cover each section. Incubate in moist chamber for 30min.	30
	b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
6. <b>Reagent 2</b> Polymer HRP anti-Mouse/Rabbit IgG (RTU)	a. Apply 2 drops (100µL) or enough volume of <b>Reagent 2</b> Polymer HRP anti-Mouse/Rabbit IgG to cover each section. Incubate in moist chamber for 15-20 min.	15-20
	b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
7. CHROMOGEN <b>Reagent 3A:</b> DAB Substrate(RTU) <b>Reagent 3B:</b> DAB Chromogen(20x)	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of <b>Reagent 3B</b> into 1mL <b>Reagent 3A</b> . Mix well. b. Apply 2 drops (100µL) or enough of DAB working solution to completely cover tissue section. Incubate for about 5 min. Monitor the color development under microscope. c. Rinse well in distill or tap water Note: Protect DAB working solution from light and use within 5 hours.	5
8. HEMATOXYLIN Supplied by user	a. Counterstain with 2 drops (100µL) or enough volume of Hematoxylin to cover tissue completely and wait about 20-30 seconds.	20 <b>seconds</b>
	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 distill 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, AP-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.

#### 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
AEC kit (20x)	C01-12	12mL	DAB+ Kit (20x)	C09-12	12mL +240mL

**Precautions:**

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

**Remarks:**

For research use only.