

## Polink-2 HRP Plus Broad DAB Detection System for Immunohistochemistry

(2-step Polymer-HRP detection system for mouse and rabbit, biotin-free,  
**Polymer Detection System with Super Sensitivity and Specificity**)

Storage: 4-8°C

Catalog No.     D41-110    110 ml (bulk, w/o chromogen)  
 D41-18    18 ml (with DAB, good for 180 slides)  
 D41-Sample    3 ml (with DAB, good for 30 slides)

### Intended Use:

Polink-2 Plus HRP Broad Detection Kit is the 3rd generation of our polymer detection systems. It uses rabbit and mouse antibody enhancer to help amplify the polymer-enzyme conjugate reaction to achieve superior sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work with antibodies. User may need to further dilute primary antibody due to super sensitivity of Polink-2 Plus detection system. It is a biotin-free system, therefore, it overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. It can be used for manual stain or autostainer. Staining conditions need to be optimized by user.

Polink-2 Plus HRP Detection System offers a wide choice for primary antibodies, including broad spectrum (for mouse and rabbit primary antibodies), mouse, rabbit, goat, and rat primary antibodies. Refer to **Related Product** section for details.

### Kit components:

Catalog No.	Product Name	Reagent 1: Rabbit and Mouse Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Rabbit and mouse (Ready-to-use)	Reagent 3A, 3B: 3A: DAB Substrate (Ready-to-use) 3B: DAB Chromogen Concentrate
D41-110	Polink-2 Plus HRP Broad for DAB Bulk Kit	110ml	110ml	Not included
D41-18	Polink-2 Plus HRP Broad with DAB Kit	18ml	18ml	30ml of DAB Substrate 3A 2ml of DAB Chromogen 3B
D41-6	Polink-2 Plus HRP Broad with DAB Kit	6ml	6ml	12ml of DAB Substrate 3A 1.5ml of DAB Chromogen 3B

### 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 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.

Reagent	Staining Procedure	Incubation Time
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. b. Rinse the slide using distilled water.	10 min.
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 3 times for 2 minutes each.	
3. Pre-antibody Blocking: Supplied by user	Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.	

4. PRIMARY ANTIBODY Supplied by user	a. Apply 2 drops (100 µl) or enough volume of PRIMARY ANTIBODY to cover the tissue section completely. Incubate in moist chamber for 30-60 min.	30-60 min.
	b. Rinse with PBS 3 times for 2 minutes each.	
5. Broad (Rabbit and Mouse) Antibody Enhancer (Ready-to-use). <b>Reagent 1</b>	a. Apply 2 drops (100 µl) or enough volume of Broad Antibody Enhancer to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS 3 times for 2 minutes each.	
6. POLYMER-HRP for Rabbit and Mouse antibody (Ready-to-use) <b>Reagent 2</b>	a. Apply 2 drops (100 µl) or enough volume of POLYMER-HRP for RABBIT and Mouse Antibody to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS for 2 min, 3 times.	
7. CHROMOGEN <b>Reagent 3A:</b> DAB Substrate <b>Reagent 3B:</b> DAB Chromogen	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B into 1ml of reagent 3A. Mix well. Protect from light and use within 5 hours. b. Apply 2 drops (100 µl) or enough to completely cover tissue, of pre-mixed DAB to each section. Incubate for about 5 min. Monitor the color development under microscope. c. Rinse with tap water for 1-2 min.	5 min.
8. HEMATOXYLIN Supplied by user	a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue completely and wait about 15-20 <b>seconds</b> . b. Rinse well under tap water for 1-2 minutes. c. Put slides in PBS until showing blue color (about 30-60 <b>seconds</b> ). d. Rinse well in distill or tap water.	15-20 <b>seconds</b>
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 AEC, AP-Red, and AP-blue. 2. O-Mount: Cat. No. E02-18 (18ml), for DAB and BCIP/NBT 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 affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting 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 Plus HRP Mouse bulk kit (without chromogen)	D37-110	110ml	Polink-2 Plus HRP Rat-NM (No cross react to mouse ) Bulk kit (without chromogen)	D46-110	110ml
Polink-2 Plus HRP Mouse DAB kit	D37-18 D37-6	18ml 6ml	Polink-2 Plus HRP Rat-NM (No cross react to mouse) DAB kit	D46-18 D46-6	18ml 6ml
Polink-2 Plus HRP Goat kit Bulk Kit (without chromogen)	D43-110	110ml	Polink-2 Plus HRP Mouse-NR (No cross react to Rat) Bulk kit (without chromogen)	D58-110	110ml
Polink-2 Plus HRP Goat DAB kit	D43-18 D43-6	18ml 6ml	Polink-2 Plus HRP Mouse-NR (No cross react to Rat) DAB kit	D58-18 D58-6	18ml 6ml
Polink-2 Plus HRP Rabbit Bulk Kit (Without chromogen)	D39-110	110ml	Polink-2 Plus HRP Broad bulk kit (without chromogen)	D41-110	110ml
Polink-2 Plus HRP Rabbit DAB Kit	D39-18 D39-6	18ml 6ml	Polink-2 Plus HRP Broad DAB kit (without chromogen)	D41-18 D41-6	18ml 6ml

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

**Remarks:** For research use only.