

Polink-2 HRP Plus Broad DAB Detection System for Immunohistochemistry

(2-step Polymer-HRP Detection System for Mouse & Rabbit Primary Antibodies)
A Biotin Free Polymer Based Detection Kit with Great Sensitivity and Specificity

Storage: 4-8°C

Catalog No. D41-125 125 ml

Intended Use:

Polink-2 Plus HRP Broad Detection Kit is the 3rd generation of polymer detection system. It uses Antibody Enhancer Broad (for rabbit and mouse) to help amplify the polymer-enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and great with difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of Polink-2 Plus detection system. This kit 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 or paraffin-embedded tissues, 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: Broad Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Rabbit and mouse (Ready-to-use)	Reagent3A, 3B: 3A: DAB Substrate (Ready-to-use) 3B: DAB Chromogen Concentrate
D41-125	Polink-2 Plus HRP DAB kit Broad Bulk kit	125ml	125ml	Reagent 3A: 125ml Reagent 3B: 7ml

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.
8. PBS-T is PBS with 0.05% Tween20

Reagent	Staining Procedure	Incubation Time
1. PEROXIDASE BLOCKING REAGENT. Supplied by user	a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H ₂ O ₂ 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-T 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. Wash with PBS –T 3 times for 2 minutes each.	
5. Broad (Rabbit and Mouse) Antibody Enhancer (Ready-to-use). Reagent 1	a. Apply 2 drops (100 µL) or enough volume of Broad Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30min. (We recommend incubating the antibody enhancer up to 30mins for best sensitivity)	10-30min.
	b. Wash with PBS-T 3 times for 2 minutes each.	
6. POLYMER-HRP for Rabbit and Mouse antibody (Ready-to-use) Reagent 2	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 10-30min. (We recommend incubating the polymer up to 30mins for best sensitivity)	10-30min.
	b. Wash with PBS for 2 min, 3 times.	
7. CHROMOGEN Reagent 3A: DAB Substrate Reagent 3B: 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 distill or 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 seconds .	15-20 seconds
	b. Rinse well under distill or tap water. 1-2 minutes.	
	c. Put slides in PBS until showing 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 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 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	DAB Kit (2-components)	C09-12	12ml +240ml
Polink-2 Plus HRP Rabbit DAB Kit	D39-18 D39-6	18ml 6ml	O-Mount (Organic)	E02-18	18ml

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

Remarks: For research use only.