Polink-2 HRP Plus Rat-NM DAB Detection System for Immunohistochemistry

(2-step Polymer-HRP detection system for rat primary antibody, biotin-free,)
Polymer Detection System with Super Sensitivity and Specificity
Clean background when detect rat antibody on mouse tissue

Storage: 4-8ºC

Catalog No.

☐	D46-110	110 ml (bulk, w/o chromogen)
☒	D46-18	18 ml (with DAB, good for 180 slides)
☐	D46-6	6 ml (with DAB, good for 50 slides)

Intended Use:

Detecting RAT primary antibody on MOUSE tissue is a very difficult task in research field due to background staining issues. Polink-2 Plus HRP Rat-NM DAB Detection Kit is specially designed to solve the problem. The secondary antibody is adsorbed to mouse and human serum proteins. This technology provides excellent specificity to detect rat primary antibody (user supplied) on mouse tissue.

Polink-2 Plus HRP Rat-NM DAB Detection Kit is the 3rd generation of polymer detection system. It uses rat antibody enhancer 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 on difficult-to-work 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:** Rat Antibody Enhancer (Ready-to-use)	**Reagent 2:** Polymer HRP for Rat (Ready-to-use)	**Reagent3A, 3B:** **3A**: DAB Substrate (Ready-to-use) **3B**: DAB Chromogen Concentrate
D46-110	Polink-2 Plus HRP Rat-NM Bulk kit for DAB	110ml	110ml	Not included
D46-18	Polink-2 Plus HRP Rat-NM DAB 18ml kit	18ml	18ml	30ml of DAB Substrate 3A 2ml of DAB Chromgen 3B
D46-6	Polink-2 Plus HRP Rat-NM DAB 6ml kit	6ml	6ml	12ml of DAB Substrate 3A 1.5ml of DAB Chromgen 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_2O_2 solution) for 10 minutes.	10 min.
	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 3 times for 2 minutes each.	

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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. Rat Antibody Enhancer (Ready-to-use). **Reagent 1**	a. Apply 2 drops (100 µL) or enough volume of Rat Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30 min. **(We recommend incubating the antibody enhancer up to 30mins for best sensitivity)**	10-30 min.
	b. Rinse with PBS 3 times for 2 minutes each.	
6. POLYMER-HRP for Rat antibody (Ready-to-use) **Reagent 2**	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for Rat Antibody to cover each section. Incubate in moist chamber for 10-30 min. **(We recommend incubating the polymer up to 30mins for best sensitivity)**	10-30 min.
	b. Rinse 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 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 tap water for 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 Broad (for mouse & rabbit) Bulk kit (without chromogen)	D41-110	110ml
Polink-2 Plus HRP Mouse DAB kit	D37-18 D37-6	18ml 6ml		Polink-2 Plus HRP Broad (for mouse & rabbit) DAB kit	D41-18 D41-6	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 or investigation only. Not for diagnostic or therapeutic use.

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