

Polink-2 AP Plus Rat-NM (No cross react to mouse) Detection System for Immunohistochemistry

(2-step Polymer-AP detection system for rat primary antibody, biotin-free.)
Polymer Detection System with Super Sensitivity and Specificity
Super clean when detecting RAT antibody on MOUSE tissue

Storage: 4-8°C

Catalog No.	<input type="checkbox"/>	D67-110	110 ml (bulk, w/o chromogen)
	<input type="checkbox"/>	D67-18	18 ml (w/o chromogen)
	<input type="checkbox"/>	D67-6	6 ml (w/o chromogen)

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 AP Rat-NM (no cross react to mouse) Detection Kit is specially designed to solve the problem. The secondary antibody is adsorbed to mouse, rabbit and human serum proteins. This technology provides excellent specificity to detect rat primary antibody (user supplied) on mouse tissue. Besides mouse tissue Polink-2 Plus AP Rat-NM Detection kit also can be used on human tissue and rabbit tissue as well.

Polink-2 Plus AP Rat-NM 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 AP 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 AP for Rat (Ready-to-use)
D67-110	Polink-2 Plus AP Rat-NM Bulk kit	110ml	110ml
D67-18	Polink-2 Plus AP Rat-NM 18ml kit	18ml	18ml
D67-6	Polink-2 Plus AP Rat-NM 6ml kit	6ml	6ml

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. 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.	
2. 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.	
3. 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.	

4. 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 min.	10 min.
	b. Rinse with PBS 3 times for 2 minutes each.	
5. POLYMER-AP for Rat antibody (Ready-to-use) Reagent 2	a. Apply 2 drops (100 µL) or enough volume of POLYMER-AP for Rat Antibody to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS for 2 min, 3 times.	
	c. Rinse with tap water.	
6. CHROMOGEN Supplied by user.	Recommended products: a. Fast-Red kit (Cat. No. C03-60) good for 600 slides b. AP-Red+ kit (Cat. No. C04-8) 40x good for 2000 slides c. BCIP/NBT (RTU) kit (Cat. No. C05-100, C05-18)	
7. 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.	
8. 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. 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:

- 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.
- 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 AP Broad Bulk kit	D68-110	110ml	Polink-2 Plus AP Mouse bulk kit	D69-110	110ml
Polink-2 Plus AP Broad 18ml kit / 6ml kit	D68-18 / D68-6	18ml / 6ml	Polink-2 Plus AP Mouse 18ml/6ml kit	D69-18 / D69-6	18ml / 6ml
Polink-2 Plus AP Rabbit bulk kit	D70-110	110ml	Fast Red Kit	C03-60	12 Tab + 60ml
Polink-2 Plus AP Rabbit 18ml kit / 6ml kit	D70-18 / D70-6	18ml / 6ml	AP-Red+ Kit (40x concentrate)	C04-8	8ml
Polink-2 Plus AP Goat bulk	D66-110	110ml	BCIP/NBT Kit	C05-100/C05-18	100ml / 18ml
Polink-2 Plus AP Goat 18ml kit / 6ml kit	D66-18 / D66-6	18ml / 6ml	GB-Mount (Aqueous)	E01-18	18ml
Polink-2 Plus AP Mouse-NR bulk Kit (no cross react to rat)	D65-110	110ml	Simpo-Mount (Aqueous)	E03-100 /E03-18	100ml / 18ml
Polink-2 Plus AP Mouse-NR 18ml kit / 6ml kit (no cross react to rat)	D65-18 / D65-6	18ml / 6ml			

Precautions: Please wear gloves and take other necessary precautions.

Remarks: For research use only.