

## Polink-2 Plus AP Rabbit Detection System for Immunohistochemistry

(2-step Polymer-AP detection system, biotin-free,)  
**Polymer Detection System with Super Sensitivity and Specificity**

Storage: 2-8°C

Catalog No.	<input type="checkbox"/>	D70-110	110 ml (bulk, w/o chromogen)
	<input type="checkbox"/>	D70-18	18 ml (w/o chromogen)
	<input type="checkbox"/>	D70-6	6 ml (w/o chromogen)

### Intended Use:

Polink-2 Plus AP Rabbit Detection Kit is the 3rd generation of polymer detection system. It uses rabbit 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: Rabbit Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer AP for Rabbit (Ready-to-use)
D70-110	Polink-2 Plus AP Rabbit bulk kit	110ml	110ml
D70-18	Polink-2 Plus AP Rabbit 18ml kit	18ml	18ml
D70-6	Polink-2 Plus AP Rabbit 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 (Min.)
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 2 min., 3 times.	
2. 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. b. Rinse with PBS for 2 min., 3 times.	30-60
3. Rabbit Antibody Enhancer (Ready-to-use). <b>Reagent 1</b>	a. Apply 2 drops (100 µL) or enough volume of RABBIT ANTIBODY ENHANCER to cover each section. Incubate in moist chamber for 10 min. b. Rinse with PBS for 2 min., 3 times.	10

4. POLYMER-AP for RABBIT (Ready-to-use) <b>Reagent 2</b>	a. Apply 2 drops (100 µL) or enough volume of POLYMER-AP for RABBIT to cover each section. Incubate in moist chamber for 10 min.	10
	b. Rinse with PBS for 2 min, 3 times.	
5. <b>Chromogen:</b> 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, C05-15)	
6. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about 20 <b>seconds</b> . b. Rinse well with tap water for 1-2 min. c. Put slides in PBS until the color turn blue (about ½ - 1 min.) d. Rinse in distill water, then rinse well with tap water	20-30 <b>seconds</b>
7. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. GB-Mount: Cat. No. E01-15 (15ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. Simpo-Mount: Cat.No. E03-15 (15ml), 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 Plus AP Broad Bulk kit	D68-110	110ml	Polink-2 Plus Mouse-NR AP bulk kit (No cross react to RAT )	D65-110	110ml
Polink-2 Plus AP Broad 18ml kit / 6ml kit	D68-18 / D68-6	18ml / 6ml	Polink-2 Plus AP Mouse-NR 18ml/6ml kit (No cross react to RAT)	D65-18 / D65-6	18ml / 6ml
Polink-2 Plus AP Mouse bulk kit	D69-110	110ml	Fast Red Kit	C03-60	12 Tab + 60ml
Polink-2 Plus AP Mouse 18ml / 6ml kit	D69-18 / 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/C05-15	100ml / 15ml
Polink-2 Plus AP Goat 18ml / 6ml kit	D66-18 / D66-6	18ml / 6ml	GB-Mount (Aqueous)	E01-15	15ml
Polink-2 Plus AP Rat-NM bulk	D67-110	110ml	Simpo-Mount (Aqueous)	E03-100 /E03-15	100ml / 15ml
Polink-2 Plus AP Rat-NM 18ml / 6ml kit	D67-18 / D67-6	18ml / 6ml			

**Precautions:** Please wear gloves and take other necessary precautions.

**Remarks:** For research use or investigation only. Not for diagnostic or therapeutic use.