

## 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.  D70-110 110 mL (Bulk, w/o chromogen)  
 D70-18 18 mL (w/ GBI-Permanent Red)  
 D70-6 6 mL (w/ GBI-Permanent Red)

### 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:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
<b>Reagent 1</b>	Rabbit Antibody Enhancer(RTU)	6mL	18mL	110mL
<b>Reagent 2</b>	Polymer AP anti-Rabbit(RTU)	6mL	18mL	110mL
<b>Reagent 3A</b>	GBI-Permanent Red Substrate (RTU)	7mL	18mL	NA
<b>Reagent 3B</b>	GBI-Permanent Red Activator (5x)	1.4mL	1.8mLx2	NA
<b>Reagent 3C</b>	GBI-Permanent Red Chromogen (100x)	70µL	180µL	NA

### Recommended Protocol:

- Fixation: To ensure the quality of the staining and obtain reproducible performance, user needs to supply appropriately fixed tissue and well prepared slides.
- Tissue needs to be adhered to the slide tightly to avoid tissue falling off.
- Paraffin embedded section must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
- Cell smear samples should be made into as thin monolayer as possible to obtain satisfactory results.
- Investigator needs to optimize dilution and incubation times for primary antibodies.
- Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
- Staining steps: DO NOT let specimen or tissue dry from this point on.
- We recommend TBS-T to be used as the wash buffer to get the highest sensitivity and clean background. Phosphate in the PBS-T may inhibit the activity of the alkaline phosphatase. **Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6.** GBI sells 10xTBS-T for your convenience (B11xx)
- Serum blocking before primary antibody incubation for GBI's Polink-1, Polink-2, and Polink-2Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent	Staining Procedure	Incubation Time
1. Alkaline Phosphatase Blocking Reagent (Not provided) We recommend using <b>GBI Dual Block E36xx</b> . Fast, easy and it will block endogenous alkaline phosphatase	a. Incubate slides in alkaline phosphatase blocking reagent. We recommend <b>GBI Dual Block E36xx</b> . b. Rinse the slide using distilled water.	Refer to datasheet
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 containing 0.05% Tween-20 or <b>1X TBS-T (See note 8 above)</b> ; 3 times for 2 minutes each.	Refer to datasheet

3. Pre-Block (Optional) Not provided	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (E07) to cover the tissue section and Incubate 10 min.	10min
	b. Drain or blot off solution. DO NOT RINSE.	
	c. See note 9 in Recommended Protocol.	
4. PRIMARY ANTIBODY Supplied by user	a. Apply 2 drops (100µL) or enough volume of PRIMARY ANTIBODY to cover the tissue section completely. Incubate in moist chamber for 30-60 min. b. Wash with PBS-T containing 0.05% Tween-20 or <b>1X TBS-T</b> ; 3 times for 2 minutes each.	30-60min
5. <b>Reagent 1</b> Rabbit Antibody Enhancer (RTU).	a. Apply 2 drops (100 µL) or enough volume of <b>Reagent 1</b> Rabbit Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30 min. <b>(We recommend incubating the antibody enhancer up to 30mins for best sensitivity)</b> b. Wash with PBS/Tween(0.05%) 2 min., 3 times.	10-30min
6. <b>Reagent 2</b> Polymer AP anti-Rabbit (RTU)	a. Apply 2 drops (100 µL) or enough volume of <b>Reagent 2</b> Polymer AP anti-Rabbit to cover each section. Incubate in moist chamber for 10-30 min. <b>(We recommend incubating the polymer up to 30mins for best sensitivity)</b> b. Wash with <b>1X TBS-T only</b> ; 3 times for 2 minutes each.	10-30min
7. <b>Reagent3A, 3B, 3C</b>  <b>Reagent 3A:</b> GBI-Permanent Red Substrate (RTU) <b>Reagent 3B:</b> GBI-Permanent Red Activator (5x) <b>Reagent 3C:</b> GBI-Permanent Red Chromogen (100x) <b>To get maximum sensitivity of AP polymer, Repeat chromogen step</b>	<b>Note:</b> Shake GBI-Permanent Red Activator before adding into GBI-Permanent Red Substrate a. Add 200µL of <b>Reagent 3B</b> (Activator) into 1mL of <b>Reagent 3A</b> (Substrate buffer) and mix well. Add 10µL of <b>Reagent 3C</b> (Chromogen) into the mixture and mix well. [Note: For fewer slides, Add 100µL of <b>Reagent 3B</b> (Activator) into 500µL of <b>Reagent 3A</b> (Substrate buffer) and mix well. Add 5µL of <b>Reagent 3C</b> (Chromogen) into the mixture and mix well. ] b. Apply 2 drops (100µL) or enough volume of GBI-Permanent Red working solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development. <b>To increase AP signal</b> aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the GBI-Permanent Red working solution to completely cover the tissue for additional <b>5 to 10min.</b> c. Rinse well with distilled water.	10min
8. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100µL) 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	20-30sec
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 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:**

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. **GBI-Permanent Red** is insoluble in organic solvent and can be coverslipped as well. however the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.

**Note: Please wipe off extra water and air dry slides before dehydration and clear.**

- a. 1x 80% Ethanol 20 seconds;
- b. 1x 95% Ethanol 20 seconds;
- c. 3x 100% Ethanol 20 seconds each;
- d. 1x 100% Xylene 20 seconds;
- e. Add 1 drop of xylene based mountant (Cat. No. O-Mount, E02-18) and coverslip. Press to push the air bubble out.

**CAUTION: DO NOT dehydrate in xylene longer than 20 seconds! It will erase GBI-Permanent Red stain!**

**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/C05-18	100ml / 18ml
Polink-2 Plus AP Goat 18ml / 6ml kit	D66-18 / D66-6	18ml / 6ml	GB-Mount (Aqueous)	E01-18	18ml
Polink-2 Plus AP Rat-NM bulk	D67-110	110ml	Simpo-Mount (Aqueous)	E03-100 /E03-18	100ml / 18ml
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 only.