Polink-2 HRP Plus Rabbit DAB Detection System for Immunohistochemistry

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

Storage: 4-8ºC

Catalog No.

☐	D39-110	110 ml (bulk, w/o chromogen)
☐	D39-18	18 ml (with DAB, good for 180 slides)
☐	D39-6	6 ml (with DAB, good for 50 slides)
☐	D39-1L	1000 ml (bulk, w/o chromogen)

Intended Use:

Polink-2 Plus HRP anti Rabbit Detection Kit is the 3rd generation of polymer detection system. It uses rabbit antibody specific 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, 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. Suitable for manual or autostain. 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:** Rabbit Antibody Enhancer (Ready-to-use)	**Reagent 2:** Polymer HRP for Rabbit (Ready-to-use)	**Reagent3A, 3B:** **3A**: DAB Substrate (Ready-to-use) **3B**: DAB Chromogen (20xConcentrate)
D39-110	Polink-2 Plus HRP DAB Rabbit Bulk kit	110ml	110ml	Not included
D39-18	Polink-2 Plus HRP DAB Rabbit 18ml kit	18ml	18ml	30ml of DAB Reagent 3A 2ml of DAB Chromgen 3B
D39-6	Polink-2 Plus HRP DAB Rabbit 6ml kit	6ml	6ml	12ml of DAB Reagent 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 as much 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. 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 (Min.)**
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
	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-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
3. Pre-Block (Optional) Supplied by user	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (E07) to cover the tissue section and Incubate 10 min.	
	b. Drain or blot off solution. DO NOT RINSE.	
	c. See note 8 in Recommended Protocol.	
4. PRIMARY ANTIBODY	a. Apply 2 drops (100 µL) or enough volume of PRIMARY ANTIBODY to cover the tissue section	30-60

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Supplied by user	completely. Incubate in moist chamber for 30-60 min.	
	b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
5. Rabbit Antibody Enhancer (Ready-to-use). **Reagent 1**	a. Apply 2 drops (100 µL) or enough volume of Rabbit 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
	b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
6. POLYMER-HRP for Rabbit (Ready-to-use) **Reagent 2**	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for RABBIT 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
	b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
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
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 show 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 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 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 Broad Bulk Kit (Without chromogen. For Ms. & Rb.)	D41-110	110ml		DAB Kit (2-components)	C09-12	12ml +240ml
Polink-2 Plus HRP Broad DAB Kit (for mouse and rabbit antibody)	D41-18 D41-6	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.

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