

## Polink-2 HRP Plus Chicken IgY DAB Detection System for Immunohistochemistry

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

Storage: 4-8°C

Catalog No.  D84-60 60 ml (bulk, w/o chromogen)  
 D84-18 18 ml (with DAB, good for 180 slides)  
 D84-sample 3 ml (with DAB, good for 25 slides)

### Intended Use:

Polink-2 Plus HRP anti Chicken IgY Detection Kit is the 3rd generation of polymer detection system. It uses Chicken IgY specific 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 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 staining and autostaining. Staining conditions need to be optimized by the user. Specificity based on ELISA and immunoelectrophoresis this kit will detect Chicken IgY light chain and whole molecule IgY. This antibody has minimal cross react with bovine, goat, guinea pig, hamster, horse, human, mouse, rabbit, rat, and sheep serum proteins but it may cross react with immunoglobulins from other species.

Polink-2 Plus HRP Detection System offers a wide choice of primary antibodies, including broad spectrum (mouse and rabbit primary antibodies), rabbit, mouse, Armenian hamster, goat, sheep, guinea pig, human and rat primary antibodies. Refer to **Related Product** section for details.

### Kit components:

Catalog No.	Product Name	Reagent 1: Chicken Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Chicken IgY (Ready-to-use)	Reagent 3A, 3B: 3A: DAB Substrate (Ready-to-use) 3B: DAB Chromogen Concentrate
D84-60	Polink-2 Plus HRP DAB Chicken IgY Bulk kit	60ml	60ml	Not included
D84-18	Polink-2 Plus HRP DAB Chicken IgY 18ml kit	18ml	18ml	30ml of DAB Reagent 3A 2ml of DAB Chromogen 3B
D84-6	Polink-2 Plus HRP DAB Chicken IgY 6ml kit	6ml	6ml	12ml of DAB Reagent 3A 1.5ml of DAB Chromogen 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.

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 <sub>2</sub> O <sub>2</sub> solution) for 10 minutes. b. Rinse the slide using distilled water.	10
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 2 min., 3 times.	
3. Blocking: optional	If blocking is needed we recommend Protein Block (Cat. No. E17-xx)	10

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. b. Rinse with PBS for 2 min., 3 times.	30-60
4. Chicken IgY Antibody Enhancer (Ready-to-use). <b>Reagent 1</b>	a. Apply 2 drops (100 µL) or enough volume of CHICKEN IgY ANTIBODY ENHANCER to cover each section. Incubate in moist chamber for 10 min. b. Rinse with PBS for 2 min., 3 times.	10
5. POLYMER-HRP for Chicken IgY (Ready-to-use) <b>Reagent 2</b>	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for Chicken IgY to cover each section. Incubate in moist chamber for 10 min. b. Rinse with PBS for 2 min, 3 times.	10
6. CHROMOGEN <b>Reagent 3A:</b> DAB Substrate <b>Reagent 3B:</b> 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
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. 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	15-20 <b>seconds</b>
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 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:

**110ml and 60ml size products are bulk kit without DAB. Go to [www.gbi-inc.com](http://www.gbi-inc.com) for details.**

Product	Cat. No.	Size	Product	Cat. No.	Size
Polink-2 Plus HRP <b>Broad</b> (anti mouse & rabbit IgG) kit	D41-110	110ml	Polink-2 Plus HRP x <b>Mouse-NR</b> IgG Kit (minimum cross react to rat) Kit	D58-110	110ml
	D41-18	18ml		D58-18	18ml
	D41-6	6ml		D58-6	6ml
Polink-2 Plus HRP x <b>Mouse</b> IgG kit	D37-110	110ml	Polink-2 Plus HRP x <b>Armenian hamster</b> IgG Kit	D82-60	60ml
	D37-18	18ml		D82-18	18ml
	D37-6	6ml		D82-6	6ml
Polink-2 Plus HRP x <b>Rabbit</b> IgG Kit	D39-110	110ml	Polink-2 Plus HRP x <b>Guinea Pig</b> IgG Kit	D83-60	60ml
	D39-18	18ml		D83-18	18ml
	D39-6	6ml		D83-6	6ml
Polink-2 Plus HRP x <b>Goat</b> IgG kit	D43-110	110ml	Polink-2 Plus HRP x <b>Sheep</b> IgG Kit	D85-60	60ml
	D43-18	18ml		D85-18	18ml
	D43-6	6ml		D85-6	6ml
Polink-2 Plus HRP x <b>Rat-NM</b> IgG kit (minimum cross react to mouse) kit	D46-110	110ml	Polink-2 Plus HRP x <b>Human</b> IgG Kit	D87-60	60ml
	D46-18	18ml		D87-18	18ml
	D46-6	6ml		D87-6	6ml

**Precautions:** DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

**Remarks:** For research use only.