Polink-2 HRP Plus Sheep IgG 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.
☒ D85-6 6 ml (with DAB, good for 60 slides)
☐ D85-18 18 ml (with DAB, good for 180 slides)
☐ D85-60 60 ml (bulk, no DAB good for 600 slides)

Intended Use:

The Polink-2 Plus HRP anti Sheep IgG Detection Kit is the 3rd generation of GBI polymer detection systems. It uses Sheep IgG 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 difficult-to-work antibodies. User may need to further dilute primary antibody due to the enhanced sensitivity of the 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 or autostaining. Staining conditions need to be optimized by the user. This kit will detect Sheep IgG light chain and whole molecule IgG with minimal cross reactivity to human serum proteins. This product may cross react with immunoglobulins from other species. **Warning: Bovine Serum Albumin (BSA) and dry milk may contain cross reactive IgG. There for we do not recommend using BSA or milk to dilute primary sheep antibodies as this may significantly increase background and reduce antibody titers.**

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

Kit components:

Catalog No.	Product Name	Reagent 1: Sheep Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Sheep antibody (Ready-to-use)	Reagent3A, 3B: **3A**: DAB Substrate (Ready-to-use) **3B**: DAB Chromogen Concentrate
D85-60	Polink-2 Plus HRP anti Sheep IgG for DAB Bulk kit	60ml	60ml	Not included
D85-18	Polink-2 Plus HRP anti Sheep IgG DAB 18ml kit	18ml	18ml	30ml of DAB Reagent 3A 2ml of DAB Chromogen 3B
D85-6	Polink-2 Plus HRP anti Sheep IgG DAB 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 needs to be adhered to the slide tightly to avoid 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 prepared as close to a 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 results: positive control, reagent control (slides treated with Isotype control reagent), and negative control.
7. Staining steps: DO NOT allow the specimen or tissue to dry at any point during the procedure.

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	

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	b. Wash with PBS 2 min., 3 times.	
3. Sheep IgG Block (optional)	Add sufficient blocking buffer to cover tissue incubate at room temperature. **Blocking step (Cat. No. E17) may improve the specificity when using sheep primary antibody. We recommend that you try staining with and without block to determine if this block will improve you're staining. Do Not** use Goat serum.	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.	30-60
	b. Rinse with PBS for 2 min., 3 times.	
4. Sheep Antibody Enhancer (Ready-to-use). **Reagent 1**	a. Apply 2 drops (100 µl) or enough volume of Sheep 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. Rinse with PBS for 2 min., 3 times.	
5. POLYMER-HRP for Sheep IgG (Ready-to-use) **Reagent 2**	a. Apply 2 drops (100 µl) or enough volume of POLYMER-HRP for Sheep IgG 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. Rinse with PBS for 2 min, 3 times.	
6. 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
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 show 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 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. The nvestigator needs to consider all factors and determines optimal conditions when interprets results.
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 for details.

Product	Cat. No.	Size		Product	Cat. No.	Size
Polink-2 Plus HRP **Broad** (anti mouse & rabbit IgG) kit	D41-110	110ml		Polink-2 Plus HRP x **Mouse-NR** 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 **Mouse** IgG kit	D37-110	110ml		Polink-2 Plus HRP x **Armenian hamster** IgG Kit	D82-60	60ml
	D37-18	18ml			D82-18	18ml
	D37-6	6ml			D82-6	6ml
Polink-2 Plus HRP x **Rabbit** IgG Kit	D39-110	110ml		Polink-2 Plus HRP x **Guinea Pig** IgG Kit	D83-60	60ml
	D39-18	18ml			D83-18	18ml
	D39-6	6ml			D83-6	6ml
Polink-2 Plus HRP x **Goat** IgG kit	D43-110	110ml		Polink-2 Plus HRP x **Chicken** IgY Kit	D84-60	60ml
	D43-18	18ml			D84-18	18ml

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	D43-6	6ml			D84-6	6ml
Polink-2 Plus HRP x **Rat**-NM IgG kit (minimum cross react to mouse) kit	D46-110	110ml		Polink-2 Plus HRP x **Human** IgG Kit	D87-60	60ml
	D46-18	18ml			D87-18	18ml
	D46-6	6ml			D87-6	6ml

Precautious: DAB may be carcinogenic. Please wear gloves and take other necessary precautions.
Remarks: For research use or investigation only. Not for diagnostic or therapeutic use.

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