GBI Labs
GOLDEN BRIDGE INTERNATIONAL

Polink-2 HRP Plus Mouse-NR (No cross react to rat) AEC Kit for Immunohistochemistry Super Sensitive to AEC Chromogen

(2-step Polymer-HRP detection system for mouse primary antibody, biotin-free,)

Polymer Detection System with Super Sensitivity and Specificity
Supper clean when using mouse antibody on rat tissue

Storage: 4-8ºC

Catalog No.
- ☐ D59-110 — 110 ml (bulk, w/o chromogen)
- ☐ D59-18 — 18 ml (with AEC, good for 180 slides)
- ☐ D59-6 — 6 ml (with AEC, good for 50 slides)

Intended Use:

Detecting MOUSE primary antibody on RAT tissue is a very difficult task in research field due to background staining issues. Polink-2 Plus HRP Mouse-NR (no cross react to rat) AEC Detection Kit is specially designed to solve the problem. The secondary antibody is adsorbed to rat, rabbit and human serum proteins. This technology provides excellent specificity to detect mouse primary antibody (user supplied) on rat tissue. Besides rat tissue Polink-2 Plus HRP Mouse-NR AEC Detection kit also can be used on human tissue and rabbit tissue as well.

Polink-2 Plus HRP Mouse-NR AEC Detection Kit is the 3rd generation of polymer detection system. It uses mouse 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 HRP AEC 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:** Mouse Antibody Enhancer (Ready-to-use)	**Reagent 2:** Polymer HRP for Mouse (Ready-to-use)	**Reagent3A, 3B, 3C:** **3A**: AEC Substrate (20x) **3B**: AEC Chromogen (20x) **3C**: Hydrogen Peroxide (20x)
D59-110	Polink-2 Plus HRP Mouse-NR Bulk kit for AEC	110ml	110ml	Not included
D59-18	Polink-2 Plus HRP Mouse-NR AEC 18ml kit	18ml	18ml	Reagent 3A: 2ml Reagent 3B: 4ml Reagent 3C: 2ml
D59-6	Polink-2 Plus HRP Mouse-NR AEC 6ml kit	6ml	6ml	Reagent 3A: 1ml Reagent 3B: 2ml Reagent 3C: 1ml

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**
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 min.
	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 3 times for 2 minutes each.	

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3. Pre-antibody Blocking: Supplied by user	Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.	
4. 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 min.
	b. Rinse with PBS 3 times for 2 minutes each.	
5. Mouse Antibody Enhancer (Ready-to-use). **Reagent 1**	a. Apply 2 drops (100 µL) or enough volume of Mouse Antibody Enhancer to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS 3 times for 2 minutes each.	
6. POLYMER-HRP for Mouse antibody (Ready-to-use) **Reagent 2**	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for Mouse Antibody to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS for 2 min, 3 times.	
7. CHROMOGEN **Reagent 3A:** AEC Substrate (20x) **Reagent 3B:** AEC Chromogen (20x) **Reagent 3C**: Hydrogen Peroxide (20x)	a. Add 1 drop of Reagent **3A** into 1ml distilled water, mix well. Then add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent **3B** and 1 drop of Reagent **3C** in the diluted substrate buffer. Mix well. Protect from light and use within 1 hour. b. Apply 2 drops (100 µL) or enough of mixture completely cover tissue. Incubate for about 5 min. Monitor the color development under microscope. c. Rinse with tap water for 1-2 min. **AEC is alcohol soluble, do not dehydrate!**	5 min.
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 showing 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. 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 affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting 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 AEC bulk kit (without chromogen)	D38-110	110ml		Polink-2 Plus HRP Broad (for mouse & rabbit) AEC Bulk kit (without chromogen)	D42-110	110ml
Polink-2 Plus HRP Mouse AEC kit	D38-18 D38-6	18ml 6ml		Polink-2 Plus HRP Broad (for mouse & rabbit) AEC kit	D42-18 D42-6	18ml 6ml
Polink-2 Plus HRP Goat AEC kit Bulk Kit (without chromogen)	D45-110	110ml		Polink-2 Plus HRP Rat-NM (No cross react to Mouse) Bulk kit (without chromogen)	D48-110	110ml
Polink-2 Plus HRP Goat AEC kit	D45-18 D45-6	18ml 6ml		Polink-2 Plus HRP Rat-NM (No cross react to Mouse) AEC kit	D48-18 D48-6	18ml 6ml
Polink-2 Plus HRP Rabbit AEC Bulk Kit (Without chromogen)	D40-110	110ml		AEC Kit (20x, 3-component)	C01-12	12ml
Polink-2 Plus HRP Rabbit AEC Kit	D40-18 D40-6	18ml 6ml		Simpo-Mount (Universal)	E03-18	18ml

Precautious: Please wear gloves and take other necessary precautions.

Remarks: For research use only.

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