

## Polink-2 Plus HRP Broad AEC (Anti-Mouse and rabbit) Detection Kit Super Sensitive to AEC Chromogen

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

Storage: 4-8°C

Catalog No.	<input type="checkbox"/>	D42-110	110 ml (bulk, w/o chromogen)
	<input type="checkbox"/>	D42-18	18 ml (with AEC, good for 180 slides)
	<input type="checkbox"/>	D42-6	6 ml (with AEC, good for 50 slides)

### Intended Use:

Polink-2 Plus HRP Broad (anti mouse and rabbit) AEC Detection Kit is the 3rd generation of polymer detection system. It uses mouse and rabbit antibody specific enhancer to help amplify the polymer-enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. This kit is extremely sensitive to AEC chromogen. 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. It can be used for manual stain or autostainer. Staining conditions need to be optimized by user. DAB chromogen is NOT recommended to use with this system.

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: Broad Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for mouse and rabbit (Ready-to-use)	Reagent 3A, 3B, 3C: 3A: AEC Substrate (20x) 3B: AEC Chromogen (20x) 3C: Hydrogen Peroxide (20x)
D42-110	Polink-2 Plus HRP Broad for AEC Bulk kit	110ml	110ml	Not included
D42-18	Polink-2 Plus HRP Broad AEC 18ml kit	18ml	18ml	Reagent 3A: 2ml Reagent 3B: 4ml Reagent 3C: 2ml
D42-6	Polink-2 Plus HRP Broad 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 <sub>2</sub> O <sub>2</sub> solution) for 10 minutes.  b. Rinse the slide using distilled water.	10 min.
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 each for 2 minutes.	
3. PRIMARY ANTIBODY	a. Apply 2 drops (100 µL) or enough volume of PRIMARY ANTIBODY to cover	30-60 min.

Supplied by user	the tissue section completely. Incubate in moist chamber for 30-60 min.	
	b. Rinse with PBS 3 times each for 2 minutes.	
4. Broad Antibody Enhancer (Ready-to-use). <b>Reagent 1</b>	a. Apply 2 drops (100 µL) or enough volume of Broad Antibody Enhancer to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS for 2 min., 3 times.	
5. POLYMER-HRP for mouse and rabbit (Ready-to-use) <b>Reagent 2</b>	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for MOUSE AND RABBIT to cover each section. Incubate in moist chamber for 10 min.	10 min.
	b. Rinse with PBS 3 times each for 2 minutes.	
6. CHROMOGEN <b>Reagent 3A:</b> AEC Substrate (20x) <b>Reagent 3B:</b> AEC Chromogen (20x) <b>Reagent 3C:</b> Hydrogen Peroxide (20x)	a. Add 1 drop of Reagent <b>3A</b> into 1ml distilled water, mix well. Then add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent <b>3B</b> and 1 drop of Reagent <b>3C</b> 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. <b>AEC is alcohol soluble, do not dehydrate!</b>	5 min.
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 <b>seconds</b> .	15-20 <b>seconds</b>
	b. Rinse well under tap water for 1-2 minutes.	
	c. Put slides in PBS until show blue color (about 30-60 <b>seconds</b> ).	
	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 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:**

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.

**Related Products:**

Product	Catalog No.	Size	Product	Catalog No.	Size
Polink-2 Plus HRP Mouse for AEC bulk kit (without chromogen)	D38-110	110ml	Polink-2 Plus HRP Rat-NM for AEC (No cross react to mouse ) Bulk kit (without chromogen)	D48-110	110ml
Polink-2 Plus HRP Mouse AEC kit	D38-18 D38-6	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 for AEC Bulk Kit (without chromogen)	D40-110	110ml	Polink-2 Plus HRP Mouse-NR (No cross react to Rat) Bulk kit (without chromogen)	D59-110	110ml
Polink-2 Plus HRP Rabbit AEC kit	D40-18 D40-6	18ml 6ml	Polink-2 Plus HRP Mouse-NR (No cross react to Rat) AEC kit	D59-18 D59-6	18ml 6ml
Polink-2 Plus HRP Goat AEC Bulk Kit (Without chromogen)	D45-110	110ml	AEC Kit (20x concentrated)	C01-12	12ml
Polink-2 Plus HRP Goat AEC Kit	D45-18 D45-6	18ml 6ml	Simpo-mount	E03-18 E03-100	18ml 100ml

**Precautious:** Please wear gloves and take other necessary precautions.

**Remarks:** For research use only.