

Polink-2 HRP Plus Rat-NM (No cross react to mouse) 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

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| Storage: 4-8°C |
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| Catalog No. | <input type="checkbox"/> | D48-110 | 110 ml (bulk, w/o chromogen) |
| | <input type="checkbox"/> | D48-18 | 18 ml (with AEC, good for 180 slides) |
| | <input type="checkbox"/> | D48-6 | 6 ml (with AEC, good for 50 slides) |
| | <input type="checkbox"/> | D48-Sample | 3 ml (with AEC, good for 25 slides) |

Intended Use:

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

Polink-2 Plus HRP Rat-NM AEC Detection Kit is the 3rd generation of polymer detection system. It uses rat 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: Rat Antibody Enhancer (Ready-to-use) | Reagent 2: Polymer HRP for Rat (Ready-to-use) | Reagent3A, 3B, 3C: 3A: AEC Substrate (20x) 3B: AEC Chromogen (20x) 3C: Hydrogen Peroxide (20x) |
|-------------|---|--|---|---|
| D48-110 | Polink-2 Plus HRP Rat-NM Bulk kit for AEC | 110ml | 110ml | Not included |
| D48-18 | Polink-2 Plus HRP Rat-NM AEC 18ml kit | 18ml | 18ml | Reagent 3A: 2ml Reagent 3B: 4ml Reagent 3C: 2ml |
| D48-6 | Polink-2 Plus HRP Rat-NM 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.
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 |
|--|--|-----------------|
| 1. PEROXIDASE BLOCKING REAGENT. Supplied by user | a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H ₂ O 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-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time. | |
| 3. Pre-Blocking (optional) | a. Add 2 (100 μL) or more drops of 10% Normal Goat Serum (E07) to cover the tissue section and | |

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| Supplied by user | Incubate 10 min. | |
| | b. Drain or blot off solution. DO NOT RINSE | |
| | c. See note 8 in Recommended Protocol. | |
| 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. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time. | |
| 5. Rat Antibody Enhancer (Ready-to-use). Reagent 1 | a. Apply 2 drops (100 µL) or enough volume of Rat 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 min. |
| | b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time. | |
| 6. POLYMER-HRP for Rat antibody (Ready-to-use) Reagent 2 | a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for Rat Antibody 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 min. |
| | b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time. | |
| 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 . 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. | 15-20 seconds |
| 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:

- 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.
- 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 Mouse-NR (No cross react to RAT) Bulk kit (without chromogen) | D59-110 | 110ml |
| Polink-2 Plus HRP Goat AEC kit | D45-18 D45-6 | 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 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 |

Precautions: Please wear gloves and take other necessary precautions.

Remarks: For research use only.