Polink-1 AP Rat-NM (No cross react to MOUSE) Detection System for IHC

(Polymer-AP detection system, biotin-free, Anti-Rat primary antibody)

Ready-to-use One Step Polymer Detection System

Clean background when detect rat antibody on mouse tissue

Storage: 4-8ºC

Catalog No.

☐	D62-110	110 mL (bulk, w/o chromogen)
☐	D62-18	18 mL (w/ chromogen)
☐	D62-6	6 mL (w/ chromogen)

Intended Use:

Detecting RAT primary antibody on MOUSE tissue is a very difficult task in research field due to background staining issues. Polink-1 AP Rat-NM 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-1 AP Rat-NM Detection Kit also can be used on human tissue as well.

Polink-1 AP Rat-NM Detection Kit is the ONE step polymer detection system that uses polymeric alkaline phosphatase (AP) -linked anti rat IgG to directly detect rat primary antibody that bound to the tissue. This technology provides excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin1. It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2nd antibody, and then streptavidin-AP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving.

Kit components:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	Polymer AP anti-Rat NM (RTU)	6mL	18mL	110mL
Reagent 2A	GBI-Permanent Red Substrate (RTU)	7mL	18mL	NA
Reagent 2B	GBI-Permanent Red Activator (5x)	1.4mL	3.6mL	NA
Reagent 2C	GBI-Permanent Red Chromogen (100x)	70µL	180µL	NA

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. Proceed IHC staining: DO NOT let specimen or tissue dry from this point on.
8. We recommend TBS-T to be used as the wash buffer to get the highest sensitivity and clean background. Phosphate in the PBS-T may inhibit the activity of the alkaline phosphatase. **Note: 1X TBS-T** =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. GBI sells 10xTBS-T for your convenience (B11xx)
9. Serum blocking before primary antibody incubation for GBI's Polink-1, Polink-2, and Polink-2 Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent	Staining Procedure	Incubation Time
1. Alkaline Phosphatase Blocking Reagent (Not provided) We recommend using **GBI Dual Block E36xx.** Fast, easy and it will block endogenous alkaline phosphatase	a. Incubate slides in alkaline phosphatase blocking reagent. We recommend **GBI Dual Block E36xx.** b. Rinse the slide using distilled water.	Refer to datasheet
2. HIER Pretreatment: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T(See note 8 above)**; 3 times for 2 minutes each.	Refer to vendor's data sheet
3. Pre-Block (Optional) Not provided	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (E07) to cover the tissue section and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE. c. See note 9 in Recommended Protocol.	10 min.
4. Primary antibody:	***Notes:*** Investigator needs to optimize dilution and incubation times	30-60 min.

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Supplied by user	a. Apply 2 (100µL) or more drops of primary antibody to cover the tissue completely. Incubate in moist chamber for 30-60 min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	
5. **Reagent:** Polymer AP anti-RAT (RTU)	a. Apply 2 (100µL) or more drops of Polymer AP anti-Rat NM to cover tissue section and Incubate in moist chamber for 15-30. **(We recommend incubating the polymer up to 30mins for best sensitivity.)** b. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	15-30 min.
6. **Reagent 2A, 2B, 2C** **Reagent 2A:** GBI-Permanent Red Substrate (RTU) **Reagent 2B:** GBI-Permanent Red Activator (5x) **Reagent 2C:** GBI-Permanent Red Chromogen (100x) **To get maximum sensitivity of AP polymer, Repeat chromogen step**	**Note:** Shake GBI-Permanent Red Activator before adding into GBI-Permanent Red Substrate. a. Add 200µL of **Reagent 2B** (Activator) into 1mL of **Reagent 2A** (Substrate buffer) and mix well. Add 10µL of **Reagent 2C**(Chromogen) into the mixture and mix well. (**Note:** For fewer slides, Add 100µL of **Reagent 2B** (Activator) into 500µL of **Reagent 2A** (Substrate buffer) and mix well. Add 5µL of **Reagent 2C**(Chromogen) into the mixture and mix well.) b. Apply 2 drops (100µL) or enough volume of GBI-Permanent Red working solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development. **To increase AP signal** aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the GBI-Permanent Red working solution to completely cover the tissue for additional **5 to 10min**. c. Rinse well with distilled water.	10 min
7. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100µL) or more drops hematoxylin to cover tissue completely and wait about 20 **seconds**. b. Rinse well with tap water for 1-2 min. c. Put slides in PBS until the color turn blue (about ½ - 1 min.) d. Rinse in distill water, then rinse well with tap water	20-30 **seconds**
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. O-Mount: Cat. No. E02-18 (18mL), for DAB and BCIP/NBT 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 affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting the result.
2. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.
5. GBI-Permanent Red is insoluble in organic solvent and can be coversliped as well. however the dehydration steps must be shorter for optimal tissue structure and chromogen signal maintenance.
   Note: Please wipe off extra water and air dry slides before dehydration and clear.
   a. 1x 80% Ethanol 20 seconds;
   b. 1x 95% Ethanol 20 seconds;
   c. 3x 100% Ethanol 20 seconds each;
   d. 1x 100% Xylene 20 seconds;
   e. Add 1 drop of xylene based mountant (Cat. No. O-Mount, E02-18) and coverslip. Press to push the air bubble out.
   CAUTION: DO NOT dehydrate in xylene longer than 20 seconds! It will erase GBI-Permanent Red stain!

Related Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
Polink-1 AP Broad Bulk kit	D17-110	110mL		**Polink-1 AP Mouse-NR Bulk kit	D57-110	110mL
Polink-1 AP Broad 18mL, 6mL Kit	D17-18 / D17-6	18mL/ 6mL		**Polink-1 AP Mouse-NR 18mL, 6mL Kit	D57-18 / D57-6	18mL / 6mL
Polink-1 AP Rabbit Bulk kit	D19-110	110mL		Fast Red Kit	C03-60	12 Tab + 60mL
Polink-1 AP Rabbit 18mL, 6mL Kit	D19-18 / D19-6	18mL/ 6mL		AP-Red+ Kit (40x concentrate)	C04-8	8mL
Polink-1 AP Goat Bulk kit	D61-110	110mL		BCIP/NBT Kit	C05-100/C05-18	100mL / 18mL
Polink-1 AP Goat 18mL, 6mL Kit	D61-18 / D61-6	18mL/ 6mL		GB-Mount (Aqueous)	E01-18	18mL
Polink-1 AP Mouse Bulk kit	D18-110	110mL		Simpo-Mount (Aqueous)	E03-100 /E03-18	100mL / 18mL
Polink-1 AP Mouse 18mL, 6mL Kit	D18-18 / D18-6	18mL/ 6mL				

**Polink -1 AP Mouse-NR kit does not cross react with Rat primary antibody

Precautious:

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Please wear gloves and take other necessary precautions.

Remarks:
For research use only.

References:
1. Bisgaard K, Pluzed KP. *Use of polymer conjugates in immunohitochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates.* Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungry, October 20-25, 1996.

2. Shi ZR. Itzkowitz SH, Kim YS. *A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcimoma tissues*. J Hitochem Cytochem 36:317-322.

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