Polink-2 AP Plus Rat-NM (No cross react to mouse) Detection System for Immunohistochemistry

(2-step Polymer-AP detection system for rat primary antibody, biotin-free,)

Polymer Detection System with Super Sensitivity and Specificity

Super clean when detecting RAT antibody on Human & Mouse Tissue

Storage: 4-8ºC

Catalog No.

☐	D67-110	110mL (Bulk, w/o chromogen)
☐	D67-18	18mL
☐	D67-6	6mL

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 AP Rat-NM (no cross react to mouse) 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.

Polink-2 Plus AP Rat-NM 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 AP 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:

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
Reagent 1	Rat-NM Antibody Enhancer(RTU)	6mL	18mL	110mL
Reagent 2	Polymer AP for Rat-NM (RTU)	6mL	18mL	110mL
Reagent 3A	GBI-Permanent Red Substrate (RTU)	7 mL	18mL	NA
Reagent 3B	GBI-Permanent Red Activator (5x)	1.4mL	2x1.8mL	NA
Reagent 3C	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 needs 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. 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:	c. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to primary antibody datasheet. d. 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 datasheet
3. Pre-Block (Optional)	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum (E07) to cover the	10min

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

Not provided	tissue section and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE. c. See note 9 in Recommended Protocol	
4. PRIMARY ANTIBODY Supplied by user	a. Apply 2 drops (100µL) or enough volume of PRIMARY ANTIBODY to cover the tissue section 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.	30-60min
5. **Reagent 1** Rat-NM Antibody Enhancer (RTU)	a. Apply 2 drops (100µL) or enough volume of **Reagent 1** Rat-NM 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)** b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** 3 times for 2 minutes each.	10-30min
6. **Reagent 2** Polymer AP for Rat-NM (RTU)	a. Apply 2 drops (100 µL) or enough volume of POLYMER-AP for Rat-NM to cover each section. Incubate in moist chamber for 10-30 min. **(We recommend incubating the polymer up to 30mins for best sensitivity)** b. Wash with **1X TBS-T only**; 3 times for 2 minutes each.	10-30min
7. **Reagent 3A, 3B, 3C** **Reagent 3A:** GBI-Permanent Red Substrate (RTU) **Reagent 3B:** GBI-Permanent Red Activator (5x) **Reagent 3C:** GBI-Permanent Red Chromogen (100x) **To get maximum sensitivity of AP polymer, Repeat chromogen step which requires you to remake the chromogen.**	**Note: Make fresh working solution and use immediately. Shake GBI-Permanent Red Activator well before adding into GBI-Permanent Red Substrate.** a. Add 200µL of **Reagent 3B** (Activator-shake well) into 1mL of **Reagent 4A** (Substrate buffer) and mix well. Add 10µL of **Reagent 3C** (Chromogen) into the mixture and mix well. [**Note:** For fewer slides, Add 100µL of **Reagent 3B** (Activator) into 500µL of **Reagent 3A** (Substrate buffer) and mix well. Add 5µL of **Reagent 3C** (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, make fresh working solution of chromogen, tap off previous chromogen, apply 2-3 drops (100µL) immediately and incubate additional 10min.** c. Rinse well with distilled water.	10min OR (10 min + 10 min)
8. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100uL) 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**
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 alcohol soluble substrates (AEC, GBI-Permanent Red and AP-Blue) 2. Simpo-Mount: Cat.No. E03-18 (18mL), 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. Investigator needs to consider all factors and determine optimal conditions when interpret 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.
3. 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.
4. **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:	Catalog No.	Size		Product	Catalog No.	Size
Polink-2 Plus AP Broad Bulk Kit	D68-110	110mL		Polink-2 Plus AP Mouse bulk Kit	D69-110	110mL
Polink-2 Plus AP Broad 18mL Kit / 6mL Kit	D68-18 / D68-6	18mL / 6mL		Polink-2 Plus AP Mouse 18mL/6mL Kit	D69-18 / D69-6	18mL / 6mL
Polink-2 Plus AP Rabbit bulk Kit	D70-110	110mL		Fast Red Kit	C03-60	60mL
Polink-2 Plus AP Rabbit 18mL Kit / 6mL Kit	D70-18 / D70-6	18mL / 6mL		AP-Red+ Kit (40x concentrate)	C04-8	8mL
Polink-2 Plus AP Goat bulk Kit	D66-110	110mL		BCIP/NBT Kit	C05-100/C05-18	100mL / 18mL
Polink-2 Plus AP Goat 18mL Kit / 6mL Kit	D66-18 / D66-6	18mL / 6mL		GB-Mount (Aqueous)	E01-18	18mL
Polink-2 Plus AP Mouse-NR bulk Kit (no cross react to rat)	D65-110	110mL		Simpo-Mount (Aqueous)	E03-100 /E03-18	100mL / 18mL
Polink-2 Plus AP Mouse-NR 18mL kit / 6mL Kit (no cross react to rat)	D65-18 / D65-6	18mL / 6mL		GBI-Permanent Red Kit	C13-18/ C13-120	18mL / 120mL

Precautious: Please wear gloves, eye protection and take other necessary precautions. If any of the reagent come in contact with skin wash area completely with plenty of water and soap. If irritation develops seek medical attention.

Remarks: For research use only.

References:

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

1. De Pasquale A, Paterlini P, Quaglino D.*Immunochemical demonstration of different antigens in single cells in paraffin-embedded histological sections.* Clin Lab Haematol. 1982;4(3):267-72.
2. Polak J. M and Van Noorden S. Introduction to Immunocytochemistry Second Edition. Bios Scientific Publishers. P41-54. 1997

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com