# Polink-2 Plus AP Human Detection System for Immunohistochemistry

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

Storage: 4-8ºC

Catalog No.
- ☐ D89-110 — 110mL (Bulk, w/o chromogen)
- ☐ D89-18 — 18mL
- ☐ D89-6 — 6mL

**Intended Use:**

The **Polink-2 Plus AP Human Kit** is designed to use with user supplied human antibody to detect human antigens on mouse tissue or cell samples. This kit has been tested in paraffin tissue. However, this kit can be used on frozen specimen and freshly prepared monolayer cell smears.
This kit is the 3rd generation of polymer detection system. Human antibody enhancer is used 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 titer primary antibody due to super sensitivity of Polink-2 Plus detection system. The **Polink-2 Plus AP Human Kit** is non-biotin system that avoids endogenous biotin non-specific binding, which is suitable for manual or autostaining. This kit is not used for screening human or humanized antibodies on human tissue.
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 | 18mLKit | 110mL Kit |
|---|---|---|---|---|
| **Reagent 1** | Human Antibody Enhancer(RTU) | 6mL | 18mL | 110mL |
| **Reagent 2** | Polymer AP for Human(RTU) | 6mL | 18mL | 110mL |
| **Reagent 3A** | GBI-Permanent Red Substrate (RTU) | 7mL | 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 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. 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 (Min.) |
|---|---|---|
| 1. Alkaline Phosphatase Blocking Reagent (Not provided)<br>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.**<br>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.<br>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)<br>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.<br>b. Drain or blot off solution. DO NOT RINSE.<br>c. See note 9 in Recommended Protocol. | 10min |



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| | | |
|---|---|---|
| 4. PRIMARY ANTIBODY<br>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.<br>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**<br>Human Antibody Enhancer (RTU) | a. Apply 2 drops (100µL) or enough volume of **Reagent 1** Human Antibody Enhancer to cover each section. Incubate in moist chamber for 10-30 min.<br>**(We recommend incubating the antibody enhancer up to 30mins for best sensitivity)**<br>b. Wash with PBS/ 0.05% Tween20 or **1xTBS-T** 3 times for 2 minutes each. | 10-30min |
| 6. **Reagent 2**<br>Polymer AP for Human (RTU) | a. Apply 2 drops (100 µL) or enough volume of POLYMER-AP for Human to cover each section. Incubate in moist chamber for 10-30 min.<br>**(We recommend incubating the polymer up to 30mins for best sensitivity)**<br>b. Wash with **1X TBS-T only**; 3 times for 2 minutes each | 10-30min |
| 7. **Reagent 3A, 3B, 3C**<br><br>**Reagent 3A:**<br>GBI-Permanent Red Substrate (RTU)<br>**Reagent 3B:**<br>GBI-Permanent Red Activator (5x<br>**Reagent 3C:**<br>GBI-Permanent Red Chromogen (100x)<br>**To get maximum sensitivity of AP polymer, Repeat chromogen step** | **Note:** Shake GBI-Permanent Red Activator before adding into GBI-Permanent Red Substrate<br>a. Add 200µL of **Reagent 3B** (Activator) into 1mL of **Reagent 3A** (Substrate) and mix well. Add 10µL of **Reagent 3C** (Chromogen) into the mixture and mix well.<br>[**Note: For fewer slides**, Add 100µL of **Reagent 3B** (Activator) into 500µL of **Reagent 3A** (Substrate) and mix well. Add 5µL of **Reagent 3C** (Chromogen) into the mixture and mix well.]<br>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**.<br>c. Rinse well with distilled water. | 10min |
| 8. Hematoxylin:<br><br>Supplied by user. | a. Counterstain with 2 (100uL) or more drops hematoxylin to cover tissue completely and wait about 20 **seconds**.<br>b. Rinse well with tap water for 1-2 min.<br>c. Put slides in PBS until the color turn blue (about ½ - 1 min.)<br>d. Rinse in distill water, then rinse well with tap water | 20-30Sec |
| 9. Mounting medium:<br><br>Supplied by user | Follow the manufacture data sheet procedure for mounting.<br>Recommended product:<br>1. GB-Mount: Cat. No. E01-18 (18mL), for alcohol soluble substrates (AEC, GBI-Permanent Red and AP-Blue)<br>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.

   **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!**



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**Related Products:**

| Product | Catalog No. | Size | | Product | Catalog No. | Size |
|---|---|---|---|---|---|---|
| Polink-2 Plus AP Broad Bulk kit | D68-110 | 110mL | | Polink-2 Plus Mouse-NR AP bulk Kit (No cross react to RAT ) | D65-110 | 110mL |
| Polink-2 Plus AP Broad 18mL Kit / 6mL Kit | D68-18 / D68-6 | 18mL / 6mL | | Polink-2 Plus AP Mouse-NR 18mL/6mL Kit (No cross react to RAT) | D65-18 / D65-6 | 18mL / 6mL |
| Polink-2 Plus AP Mouse Bulk kit | D69-110 | 110mL | | Fast Red Kit | C03-60 | 60mL |
| Polink-2 Plus AP Mouse 18ml kit / 6ml Kit | D69-18 / D68-6 | 18mL / 6mL | | AP-Red+ Kit (40x concentrate) | C04-8 | 8mL |
| Polink-2 Plus AP Rabbit bulk Kit | D70-110 | 110mL | | BCIP/NBT Kit | C05-100/C05-18 | 100mL / 18mL |
| Polink-2 Plus AP Rabbit 18mL Kit / 6mL Kit | D70-18 / D70-6 | 18mL / 6mL | | GB-Mount (Aqueous) | E01-18 | 18mL |
| Polink-2 Plus AP Goat Bulk Kit | D66-110 | 110mL | | Simpo-Mount (Aqueous) | E03-100 /E03-18 | 100mL / 18mL |
| Polink-2 Plus AP Goat 18mL Kit / 6mL Kit | D66-18 / D66-6 | 18mL / 6mL | | GBI-Permanent Red Kit | C13-18/ C13-120 | 18mL / 120mL |
| Polink-2 Plus AP Rat-NM (no cross react to mouse) Bulk Kit | D67-110 | 110mL | | | | |
| Polink-2 Plus AP Rat-NM (no cross react to mouse) 18mL kit / 6mL Kit | D67-18 / D67-6 | 18mL / 6mL | | | | |

**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:**
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



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