



PolyStain 2-Step Plus Kit –  
HRP for DAB Detection  
System for Sheep  
Antibody

---

**NB-23-00057-1 (60mL, no chromogen)**

**NB-23-00057-2 (18ml, with DAB)**

**NB-23-00057-3 (6ml, with DAB)**

## ***PolyStain 2-Step Plus Kit - HRP Sheep DAB Detection System for Immunohistochemistry***

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

*NB-23-00056-1                    size : 60mL, no chromogen*  
*NB-23-00056-2            size : 18ml, with DAB (good for 180 slides)*  
*NB-23-00056-3            size : 6ml, with DAB (good for 50 slides)*

### **Intended Use:**

The PolyStain 2-Step Plus HRP anti Sheep IgG Detection Kit is the 3rd generation of Neo Biotech polymer detection systems. It uses Sheep IgG specific 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 difficult-to-work antibodies. User may need to further dilute primary antibody due to the enhanced sensitivity of the PolyStain 2-Step Plus detection system. It is a biotinfree system; therefore, it overcomes the non-specific staining caused by 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 staining or autostaining. Staining conditions need to be optimized by the user. This kit will detect Sheep IgG light chain and whole molecule IgG with minimal cross reactivity to human serum proteins. This product may cross react with immunoglobulins from other species.

**Warning: Bovine Serum Albumin (BSA) and dry milk may contain cross reactive IgG. There for we do not recommend using BSA or milk to dilute primary sheep antibodies as this may significantly increase background and reduce antibody titers.**

PolyStain 2-Step Plus HRP Detection Systems offer a wide variety of primary antibodies, including broad spectrum (mouse and rabbit primary antibodies), rabbit, mouse, hamster, goat, chicken, guinea pig, human and rat primary antibodies. Refer to the **Related Product** section for details

### **Kit Components:**

| Catalog No.   | Product Name                                       | Reagent 1:<br>Sheep Antibody Enhancer (RTU) | Reagent 2:<br>Polymer HRP for Sheep (RTU) | Reagent 3A, 3B:<br>3A: DAB Substrate (RTU)<br>3B: DAB Chromogen Concentrate |
|---------------|--|---|---|---|
| NB-23-00057-1 | PolyStain 2-Step Plus Kit - HRP for DAB Sheep Bulk | 60ml  | 60ml                                      | Not included  |
| NB-23-00057-2 | PolyStain 2-Step Plus Kit - HRP for DAB Sheep 18ml | 18ml  | 18ml                                      | 30ml of DAB Reagent 3A<br>2ml of DAB Chromgen 3B                            |
| NB-23-00057-3 | PolyStain 2-Step Plus Kit - HRP for DAB Sheep 6ml  | 6ml   | 6ml                                       | 12ml of DAB Reagent 3A<br>1.5ml of DAB Chromgen 3B                          |

### **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. Staining steps: DO NOT let specimen or tissue dry from this point on.

## Reagent:

| Reagent  | Staining Procedure  | Incubation Time (Min.)  |
|--|---|-------------------------|
| 1. PEROXIDASE BLOCKING REAGENT. Supplied by user                                     | <ol style="list-style-type: none"> <li>a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H<sub>2</sub>O<sub>2</sub> solution) for 10 minutes.</li> <li>b. Rinse the slide using distilled water.</li> </ol>  | 10                      |
| 2. HIER PRETREATMENT:  | <ol style="list-style-type: none"> <li>a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. Please check the data sheet of primary antibody</li> <li>b. Wash with PBS 2 min., 3 times.</li> </ol>   |                         |
| 3. Sheep IgG Block (optional)  | Add sufficient blocking buffer to cover tissue incubate at room temperature. <b>Blocking step</b> (Cat.# NB-23-00179-1 100ml / -2 18ml) <b>may improve the specificity when using sheep primary antibody. We recommend that you try staining with and without block to determine if this block will improve you're staining.</b>  |                         |
| 4. PRIMARY ANTIBODY<br>Supplied by user  | <ol style="list-style-type: none"> <li>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.</li> <li>b. Rinse with PBS for 2 min., 3 times.</li> </ol>   | 30-60                   |
| 5. Sheep Antibody Enhancer<br>(Ready-to-use).<br><b>Reagent 1</b>                    | <ol style="list-style-type: none"> <li>a. Apply 2 drops (100 µL) or enough volume of Sheep ANTIBODY ENHANCER to cover each section. Incubate in moist chamber for 10 min.</li> <li>b. Rinse with PBS for 2 min., 3 times.</li> </ol>  | 10                      |
| 6. POLYMER-HRP for Sheep IgY (Ready-to-use)<br><b>Reagent 2</b>                      | <ol style="list-style-type: none"> <li>a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP to cover each section.</li> <li>b. Incubate in moist chamber for 10 min.</li> <li>c. Rinse with PBS for 2 min, 3 times.</li> </ol>   | 10                      |
| 7. CHROMOGEN<br><b>Reagent 3A:</b> DAB Substrate<br><b>Reagent 3B:</b> DAB Chromogen | <ol style="list-style-type: none"> <li>a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B into 1ml of reagent 3A. Mix well. Protect from light and use within 5 hours.</li> <li>b. Apply 2 drops (100 µL) or enough to completely cover tissue, of pre-mixed DAB to each section. Incubate for about 5 min. Monitor the color development under microscope.</li> <li>c. Rinse with tap water for 1- 2 min.</li> </ol> | 5                       |
| 8. HEMATOXYLIN<br>Supplied by user   | <ol style="list-style-type: none"> <li>a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue completely and wait about 15-20 seconds.</li> <li>b. Rinse well under tap water for 1-2 minutes.</li> <li>c. Put slides in PBS until show blue color (about 30-60 seconds).</li> <li>d. Rinse well in distill or tap water</li> </ol>  | 15-20<br><b>seconds</b> |

|   |   |                        |
|---|---|------------------------|
| <p>9. Mounting medium:<br/>Supplied by user</p> | <p>Follow the manufacture data sheet procedure for mounting.<br/>Recommended product:</p> <ol style="list-style-type: none"> <li>NeoBio Mount AQ (Cat# NB-23-00155-3, 18ml) for AEC, AP-Red, and AP-blue.</li> <li>NeoBio Mount Perm (Cat.# NB-23-00156, 18ml), for DAB</li> <li>NeoBio Mount Universal (Cat.# NB-23-00157-2, 18ml or NB-23-00157-1, 100ml), universal permanent mounting medium. Can be used with or without cover slip</li> </ol> | <p>Refer to insert</p> |
|---|---|------------------------|

### 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. We recommend NeoBlock, NB-23-00169-1 (500ml), NB-23-00169-2 (100ml), NB-23-00169-3 (18ml)

### Precautions:

DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

### Remarks:

For research use only.

### Storage:

Store at 4°C.

### Related products

| Product  | Catalog No.        | Size         |
|--|--------------------|--------------|
| PolyStain 2-Step Plus Kit - HRP Mouse bulk kit (without chromogen)                       | NB-23-00049-2      | 110ml        |
| PolyStain 2-Step Plus Kit - HRP Mouse DAB kit  | NB-23-00049-3 / -4 | 18ml<br>6ml  |
| PolyStain 2-Step Plus Kit - HRP Goat kit Bulk Kit (without chromogen)                    | NB-23-00051-2      | 110ml        |
| PolyStain 2-Step Plus Kit - HRP Goat DAB kit   | NB-23-00051-3 / -4 | 18ml<br>6ml  |
| PolyStain 2-Step Plus Kit - HRP Broad Bulk Kit (Without chromogen, for Ms. & Rb.)        | NB-23-00048-3      | 110ml        |
| PolyStain 2-Step Plus Kit - HRP Broad DAB Kit (for mouse and rabbit antibody)            | NB-23-00048-4 / -5 | 18ml<br>6ml  |
| PolyStain 2-Step Plus Kit - HRP Rat-NM (No cross to Mouse ) Bulk kit (without chromogen) | NB-23-00052-1      | 110ml        |
| PolyStain 2-Step Plus Kit - HRP Rat-NM (No cross to Mouse) DAB kit                       | NB-23-00052-2 / -3 | 18ml<br>6ml  |
| PolyStain 2-Step Plus Kit - HRP Mouse-NR (No cross to Rat) Bulk kit (without chromogen)  | NB-23-00053-1      | 110ml        |
| PolyStain 2-Step Plus Kit - HRP Mouse-NR (No cross to Rat) DAB kit                       | NB-23-00053-2 / -3 | 18ml<br>6ml  |
| DAB Kit (2-components)   | NB-23-000148       | 12ml + 240ml |
| NeoBio Mount Perm  | NB-23-000148       | 18ml         |