



NeoStain Poly 2-Step  
Plus Kit

NB-23-00055 -1

NB-23-00055 -2

NB-23-00055 -3

### NeoStain Poly 2-Step Plus Kit

#Cat: NB-23-00055-1	Size: 60mL (bulk, w/o chromogen)
#Cat: NB-23-00055-2	Size: 18mL (with DAB, good for 180 slides)
#Cat: NB-23-00055-3	Size: 6mL (with DAB, good for 50 slides)

#### Intended Use:

Storage: 4-8°C

NeoStain Poly 2-Step Plus Kit is the 3rd generation of polymer detection system. It uses Guinea Pig 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 on difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of the NeoStain Poly 2-Step Plus detection system. It is a biotin-free system, overcoming 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 or autostaining. Staining conditions need to be optimized by the user. This kit will detect guinea pig light chain and whole molecule IgG, and may cross react with other species.

NeoStain Poly 2-Step Plus Kit, HRP Detection System offers a wide variety of primary antibodies, including broad spectrum (mouse and rabbit primary antibodies), rabbit, mouse, Armenian hamster, goat, sheep, chicken, human and rat primary antibodies. Refer to Related Product section for details.

#### Kit components:

Component No	Product Name	Reagent 1: Guinea Pig Antibody Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Guinea Pig antibody (Ready-to-use)	Reagent3A, 3B: 3A: DAB Substrate (Ready-to-use) 3B: DAB Chromogen Concentrate
NB-23-00055-1	NeoStain Poly 2-Step Plus Kit, HRP for DAB, Guinea Pig, no chromogen	60ml	60ml	Not included
NB-23-00055-2	NeoStain Poly 2-Step Plus Kit, HRP, Guinea Pig, with DAB	18ml	18mL	30ml of DAB Reagent 3A 2ml of DAB Chromogen 3B
NB-23-00055-3	NeoStain Poly 2-Step Plus Kit, HRP DA	6ml	6mL	12ml of DAB Reagent 3A 1.5ml of DAB Chromogen 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. Tissues need to be adhered to the slide tightly to avoid falling off.
3. Paraffin embedded sections must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be prepared as close to a 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 control, reagent control (slides treated with Isotype control reagent), and negative control.
7. Staining steps: DO NOT allow the specimen or tissue to dry at any point during the procedure.

Reagent	Staining Procedure	Staining Procedure
1. Peroxidase Blocking Reagent. Supplied By User	a. Incubate slides in Peroxidase Blocking Reagent (Ready-to-use 3% H <sub>2</sub> O <sub>2</sub> solution) for 10 minutes b. Rinse the slide using distilled water.	10
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 2 min., 3 times.	
3. Blocking: optional	If blocking is needed, we recommend Protein Block (Cat. No. NB-23-00179)	10
3. 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 b. Rinse with PBS for 2 min., 3 times.	30-60
4. Guinea Pig Antibody Enhancer (Ready-to-use). Reagent 1	a. Apply 2 drops (100 µL) or enough volume of Guinea Pig 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. Rinse with PBS for 2 min., 3 times	10-30
5. Polymer-Hrp for Guinea Pig (Ready-to-use) Reagent 2	a. Apply 2 drops (100 µL) or enough volume of Polymer-Hrp for Guinea Pig to cover each section. Incubate in moist chamber for 10-30 min. (We recommend incubating the polymer up to 30mins for best sensitivity) b. Rinse with PBS for 2 min, 3 times.	10-30
6. Chromogen Reagent 3A: DAB Substrate	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. 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. c. Rinse with tap water for 1-2 min.	5

<p>7. Hematoxylin Supplied by user</p>	<p>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 show blue color (about 30-60 seconds). d. Rinse well in distill or tap water</p>	<p>15-20 seconds</p>
<p>8. Mounting medium: Supplied by user</p>	<p>Follow the manufacture data sheet procedure for mounting. Recommended product: 1. NeoBio Mount AQ (Aqueous solution, use with AEC and AP-Red): Cat. No. NB-23-00155-3 (18ml), for AEC, AP-Red, and AP-blue. 2. NeoBio Mount Perm (Permanent mount for DAB, BCIP/NBT): Cat. No. NB-23-00156 (18ml), for DAB 3. NeoBio Mount Universal (Water Based, universal) Kit: Cat.No. NB-23-00157-2 (18ml), or NB-23-00157-1 (100ml), universal permanent mounting medium. Can be used with or without cover slip</p>	<p>Refer to insert</p>

**Protocol Notes:**

1. The fixation, tissue slide thickness, primary antibody dilution and incubation time may effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting results.
2. Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.

## Related Products:

110ml and 60ml size products are bulk kit without DAB. Go to [www.neo-biotech.com](http://www.neo-biotech.com) for details.

Product	Catalog No.	Size
NeoStain Poly 2-Step Plus Kit Broad (anti mouse & rabbit IgG) kit	NB-23-00048-3	110ml
	NB-23-00048-4	18ml
	NB-23-00048-5	6ml
NeoStain Poly 2-Step Plus Kit x Mouse IgG kit	NB-23-00049-2	110ml
	NB-23-00049-3	18ml
	NB-23-00049-4	6ml
NeoStain Poly 2-Step Plus Kit x Rabbit IgG Kit	NB-23-00050-2	110ml
	NB-23-00050-3	18ml
	NB-23-00050-4	6ml
NeoStain Poly 2-Step Plus Kit x Goat IgG kit	NB-23-00051-2	110ml
	NB-23-00051-3	18ml
	NB-23-00051-4	6ml
NeoStain Poly 2-Step Plus Kit x Rat-NM IgG kit (minimum cross react to mouse) kit	NB-23-00052-1	110ml
	NB-23-00052-2	18ml
	NB-23-00052-3	6ml
NeoStain Poly 2-Step Plus Kit x Mouse-NR IgG Kit (minimum cross react to rat) Kit	NB-23-00053-1	110ml
	NB-23-00053-2	18ml
	NB-23-00053-3	6ml
NeoStain Poly 2-Step Plus Kit x Chicken IgY Kit	NB-23-00056-1	60ml
	NB-23-00056-2	18ml
	NB-23-00056-3	6ml
NeoStain Poly 2-Step Plus Kit x Sheep IgG Kit	NB-23-00057-1	60ml
	NB-23-00057-2	18ml
	NB-23-00057-3	6ml
NeoStain Poly 2-Step Plus Kit x Human IgG Kit	NB-23-00054-1	60ml
	NB-23-00054-2	18ml
	NB-23-00054-3	6ml

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

**Remarks:** For research use only.