



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

NB-23-00055-1 (60mL, no chromogen)

NB-23-00055-2 (18ml, with DAB)

NB-23-00055-3 (6ml, with DAB)

PolyStain 2-Step Plus Kit - HRP Guinea Pig DAB Detection System for Immunohistochemistry

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

NB-23-00055-1 size : 60mL, no chromogen
 NB-23-00055-2 size : 18ml, with DAB (good for 180 slides)
 NB-23-00055-3 size : 6ml, with DAB (good for 50 slides)

Intended Use:

The PolyStain 2-Step Plus HRP anti Guinea Pig Detection Kit is the 3rd generation of Neo Biotech 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 PolyStain 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.

The PolyStain 2-Step Plus 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:

Catalog No.	Product Name	Reagent 1: Guinea Pig Antibody Enhancer (RTU)	Reagent 2: Polymer HRP for Guinea Pig (RTU)	Reagent 3A, 3B: 3A: DAB Substrate (RTU) 3B: DAB Chromogen Concentrate
NB-23-00055-1	PolyStain 2-Step Plus Kit - HRP for DAB G.Pig Bulk	60ml	60ml	Not included
NB-23-00055-2	PolyStain 2-Step Plus Kit - HRP for DAB G.Pig 18ml	18ml	18ml	30ml of DAB Reagent 3A 2ml of DAB Chromgen 3B
NB-23-00055-3	PolyStain 2-Step Plus Kit - HRP for DAB G.Pig 6ml	6ml	6ml	12ml of DAB Reagent 3A 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"> a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H₂O₂ solution) for 10 minutes. b. Rinse the slide using distilled water. 	10
2. HIER PRETREATMENT:	<ol style="list-style-type: none"> 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. Pre-antibody Blocking: Supplied by user	Pre-antibody blocking is optional and can be omitted if primary antibodies are diluted in buffers containing 2-10% normal goat serum.	
4. PRIMARY ANTIBODY Supplied by user	<ol style="list-style-type: none"> 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
5. Guinea Pig Antibody Enhancer (Ready-to-use). Reagent 1	<ol style="list-style-type: none"> a. Apply 2 drops (100 µL) or enough volume of GUINEA PIG ANTIBODY ENHANCER to cover each section. Incubate in moist chamber for 10 min. b. Rinse with PBS for 2 min., 3 times. 	10
6. POLYMER-HRP for Guinea Pig (Ready-to-use) Reagent 2	<ol style="list-style-type: none"> a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP to cover each section. b. Incubate in moist chamber for 10 min. c. Rinse with PBS for 2 min, 3 times. 	10
7. CHROMOGEN Reagent 3A: DAB Substrate Reagent 3B: DAB Chromogen	<ol style="list-style-type: none"> 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
8. HEMATOXYLIN Supplied by user	<ol style="list-style-type: none"> 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 	15-20 seconds
9. Mounting medium: Supplied by user	<p>Follow the manufacture data sheet procedure for mounting. Recommended product:</p> <ol style="list-style-type: none"> a. NeoBio Mount AQ (Cat# NB-23-00155-3, 18ml) for AEC, AP-Red, and AP-blue. b. NeoBio Mount Perm (Cat.# NB-23-00156, 18ml), for DAB c. 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 	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. 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 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 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 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 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 6ml
DAB Kit (2-components)	NB-23-000148	12ml + 240ml
NeoBio Mount Perm	NB-23-000148	18ml