



PolyStain 2-Step Plus Kit, HRP for DAB, Syrian Hamster

2-Step Polymer-HRP Detection System, biotin-free

NB-23-00058-1 (60ml)

NB-23-00058-2 (18ml)

NB-23-00058-3 (6ml)



PolyStain 2-Step Plus Kit, HRP for DAB, Syrian Hamster

NB-23-00058-1; NB-23-00058-2; NB-23-00058-3

Storage: 4-8°C

INTENDED USE:

PolyStain 2-Step Plus Kit, HRP for DAB, Syrian Hamster Detection Kit is the 3rd generation of IHC polymer kits design. This kit uses Syrian Hamster specific antibody enhancer to help amplify the polymer-enzyme conjugate reaction to achieve excellent sensitivity and specificity. Our kits produce consistent immunostaining outcomes on archival tissues and with difficult antibodies. As with all PolyStain 2-Step Plus Kit, this kit is a biotin-free system; therefore, it overcomes the non-specific staining caused by endogenous biotin. Syrian Hamster PolyStain 2-Step Plus Kits are recommended for frozen or paraffin-embedded tissue, freshly prepared lymphocytes, and fixed culture cells. This kit can be used for manual IHC staining or IHC on an autostainer. User may need to further dilute primary antibody due to the sensitivity of PolyStain 2-Step Plus Kit detection system. Syrian hamster PolyStain 2-Step Plus Kits NB-23-00058 has been screened on human, mouse, and rat paraffin embedded tissue and found to have no background. If you are using Armenian Hamster primary antibody, we recommend you use PolyStain 2-Step Plus Kit, NB-23-00054 as this kit will not work for detection of Armenian Hamster primary antibodies. PolyStain 2-Step Plus Kit System offers a wide choice 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:	Reagent 2:	Reagent3A, 3B:
	PolyStain 2-Step Plus Kit, HRP for DAB, Syrian Hamster	Syrian Hamster Antibody Enhancer (RTU)	Polymer HRP for Syrian Hamster (RTU)	3A: DAB Substrate (RTU) 3B: DAB Chromogen Concentrate
NB-23-00058-1	No Chromogen	60mL	60mL	Not included
NB-23-00058-2	With DAB	18mL	18mL	30ml of DAB Reagent 3A 2ml of DAB Chromogen 3B
NB-23-00058-3	With DAB	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 tissues and well prepared slide sections.
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 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 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	Staining Procedure	Incubation Time (Min.)
1. Peroxidase blocking reagent: Supplied by user.	a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H ₂ O ₂ solution) for 10 minutes. b. Rinse the slide using distilled water.	10 min.
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.	
Blocking: optional	If blocking is needed we recommend Protein Block (Cat. No. NB-23-00179)	10min
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 min.
4. Syrian Hamster Antibody Enhancer (Ready-to-use). Reagent 1	a. Apply 2 drops (100 µL) or enough volume of Syrian Hamster 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	10-60 min

<p>5. POLYMER-HRP for Syrian Hamster (Ready-to-use)</p> <p>Reagent 2</p>	<p>a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for Syrian Hamster to cover each section. Incubate in moist chamber for 10-30 min.</p> <p>(We recommend incubating the polymer up to 30mins for best sensitivity)</p> <p>b. Rinse with PBS for 2 min, 3 times.</p>	<p>10 -30 min</p>
<p>6. CHROMOGEN</p> <p>Reagent 3A: DAB Substrate</p> <p>Reagent 3B: DAB Chromogen</p>	<p>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 5-7 hours.</p> <p>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.</p> <p>c. Rinse with tap water for 1-2 min.</p>	<p>5 min</p>
<p>7. HEMATOXYLIN</p> <p>Supplied by user</p>	<p>a. Counterstain with 2 drops (100 µl) or enough volume of Hematoxylin to cover tissue completely and wait about 15-20 seconds.</p> <p>b. Rinse well under tap water for 1-2 minutes</p> <p>c. Put slides in PBS until show blue color (about 30-60 seconds).</p> <p>d. Rinse well in distill or tap water</p>	<p>15-20 seconds</p>
<p>9. Mounting media:</p> <p>Supplied by user</p>	<p>We recommend an organic mounting media but you can use and aqueous mount. Follow the manufacture data sheet procedure for mounting. Recommended product:</p> <ol style="list-style-type: none"> 1. NeoMount AQ: Cat. No. NB-23-00155-3 (18mL), for Aqueous solution, use with AEC and AP-Red 2. NeoMount Perm: Cat. No. NB-23-00156 (18mL), for DAB,BCIP/NBT 3. NeoMount Universal: Cat. No. NB-23-00157-2 (18mL), or NB-23-00157-1 (100mL), Water Based, universal <p>Can be used with or without cover slip</p>	<p>Refer to insert</p>

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 interpreting result.
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:

Product	Catalog No	Size	Product	Catalog No	Size
PolyStain 2-Step Plus Kit, HRP for DAB, Mouse & Rabbit,	<u>NB-23-00048-3</u> <u>NB-23-00048-4</u> <u>NB-23-00048-5</u>	<u>110 ml</u> <u>18ml</u> <u>6ml</u>	PolyStain 2-Step Plus Kit, HRP for DAB, Mouse-NR	<u>NB-23-00053-1</u> <u>NB-23-00053-2</u> <u>NB-23-00053-3</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>
PolyStain 2-Step Plus Kit, HRP for DAB, Mouse	<u>NB-23-00049-2</u> <u>NB-23-00049-3</u> <u>NB-23-00049-4</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>	PolyStain 2-Step Plus Kit, HRP for DAB, Sheep	<u>NB-23-00057-1</u> <u>NB-23-00057-2</u> <u>NB-23-00057-3</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>
PolyStain 2-Step Plus Kit, HRP for DAB, Rabbit	<u>NB-23-00050-2</u> <u>NB-23-00050-3</u> <u>NB-23-00050-4</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>	PolyStain 2-Step Plus Kit, HRP for DAB, Guinea Pig	<u>NB-23-00055-1</u> <u>NB-23-00055-2</u> <u>NB-23-00055-3</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>
PolyStain 2-Step Plus Kit, HRP for DAB, Goat	<u>NB-23-00051-2</u> <u>NB-23-00051-3</u> <u>NB-23-00051-4</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>	PolyStain 2-Step Plus Kit, HRP for DAB, Chicken	<u>NB-23-00056-1</u> <u>NB-23-00056-2</u> <u>NB-23-00056-3</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>
PolyStain 2-Step Plus Kit, HRP for DAB, Rat-NM	<u>NB-23-00052-1</u> <u>NB-23-00052-2</u> <u>NB-23-00052-3</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>	PolyStain 2-Step Plus Kit, HRP for DAB, Armenian Hamster	<u>NB-23-00054-1</u> <u>NB-23-00054-2</u> <u>NB-23-00054-3</u>	<u>110ml</u> <u>18ml</u> <u>6ml</u>

PRECAUTION:

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

For research use only.



