



NeoLink-2 HRP Broad  
Spectrum for DAB kit

NB-23-00045

## NeoLink-2 HRP Broad Spectrum for DAB kit

#Cat: NB-23-00045-6	Size: 110ml, no chromogen
#Cat: NB-23-00045-7	Size: 60ml, no chromogen
#Cat: NB-23-00045-8	Size: 60ml, with DAB
#Cat: NB-23-00045-9	Size: 18ml, with DAB
#Cat: NB-23-00045-10	Size: 6ml, with DAB

### Intended Use

NeoLink-2 HRP DAB Broad-2 Detection Kit is a HRP polymer based detection system that can be used to detect mouse or rabbit primary antibodies for immunohistochemical screens of human tissues. NeoLink-2 kits are a second generation polymer detection system that uses polymer helper and polymeric HRP-linked antibody conjugates to get consistent immunostaining outcomes on archival tissues and to increase sensitivity on difficult-to-work antibodies, whether the antigen is cytoplasmic, membranous, or nuclear. This technology provides excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. This kit has been tested on paraffin embedded tissue only; however it may be used on frozen or freshly prepared monolayer cell smears.

### Kit Component

Component No.	Content	NB-23-00045-10 6mL kit	NB-23-00045-9 18ml Kit	NB-23-00045-8 60D ml Kit	NB-23-00045-7 60ml Kit	NB-23-00045-6 110ml Kit
<b>Reagent 1</b>	Polymer Helper (RTU)	6 ml	18 ml	60 ml	60 ml	110 ml
<b>Reagent 2</b>	Polymer HRP anti-Mouse/Rabbit IgG (RTU)	6 ml	18 ml	60 ml	60 ml	110 ml
<b>Reagent 3A</b>	DAB Substrate (RTU)	12 ml	15ml x 2	60 ml	NA	NA
<b>Reagent 3B</b>	DAB Chromogen (20x)	1.5 ml	2 ml	3 ml	NA	NA

### Storage

Store at 4-8°C

## 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.
8. Serum blocking before primary antibody incubation for Neo Biotech's NeoLink-1, NeoLink-2, and NeoLink-2Plus is not required because all our antibody conjugates are absorbed to human serum.

Reagent	Staining Procedure	Incubation Time (Min.)
<b>1. Peroxidase Blocking Reagent</b> 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 min. b. Rinse the slide using distilled water.	10
<b>2. HIER Pretreatment</b>	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-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
<b>3. Pre-Block (Optional)</b> Not provided	a. Add 2 (100 µL) or enough volume of 10% Normal Goat Serum (NB-23-00169) to cover the tissue section and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE. c. See note 8 in Recommended Protocol.	10

<p><b>4. Primary antibody:</b> Supplied by user</p>	<p>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. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time</p>	<p>30-60</p>
<p><b>5. Reagent 1:</b> HRP Polymer</p>	<p>a. Apply 2 drops (100 µL) or enough volume of <b>Reagent 1</b> Polymer Helper to cover each section. Incubate in moist chamber for 15-30 min. <b>(We recommend incubating the polymer helperup to 30mins for best sensitivity)</b> b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.</p>	<p>15-30</p>
<p><b>6. Reagents 2</b> Polymer HRP anti-Mouse/Rabbit IgG (RTU)</p>	<p>a. Apply 2 drops (100µL) or enough volume of Reagent 2 Polymer HRP anti-Mouse/Rabbit IgG to cover each section. Incubate in moist chamber for 30min. b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.</p>	<p>30</p>
<p><b>7. CHROMOGEN</b> Reagent 3A: DAB Substrate(RTU) Reagent 3B: DAB Chromogen</p>	<p>a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B into 1mL Reagent 3A. Mix well. b. Apply 2 drops (100µL) or enough of DAB working solution to completely cover tissue section. Incubate for about 5 min. Monitor the color development under microscope. c. Rinse well in distill or tap water Note: Protect DAB working solution from light and use within 5 hours.</p>	<p>5</p>

<b>8. Hematoxylin: Supplied by user.</b>	<p>a. Counterstain with 2 drops (100 ul) or enough volume of hematoxylin to cover tissue completely and wait about 20-30 seconds.</p> <p>b. Rinse well with tap water for 1-2 min.</p> <p>c. Put slides in PBS until the color turn blue (about 30-60 seconds.)</p> <p>d. Rinse well in distill or tap water</p>	<p>20 seconds</p>
<b>9. Mounting medium: Supplied by user</b>	<p>Follow the manufacture data sheet procedure for mounting.</p> <p>Recommended product:</p> <ol style="list-style-type: none"> <li>1. Neo-Mount: cat. No NB-23-00155-3 (18ml), for alcohol soluble substrates (AEC, AP-Red, and AP-blue)</li> <li>2. O-Mount: Cat. No. NB-23-00156 (18 ml), for DAB.</li> <li>3. Simpo-Mount/ 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.</li> </ol>	<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 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.

**Related Products:**

Product	Catalog No.	Size	Product	Catalog No.	size
NeoStain Poly 2-Step Kit, AP, Mouse & Rabbit, no chromogen	NB-23-00047-1	110 ml	AP-Red concentrated (40x) kit for 3000 slides	NB-23-00143	8 ml
NeoStain Poly 2-Step Kit, AP, Mouse & Rabbit, with Permanent Red	NB-23-00047-2 / NB-23-00047-3	18ml / 6ml	BCIP/NBT (RTU)	NB-23-00144-1 / NB-23-00144-2	100ml / 18 ml
NeoStain Poly 2-Step Kit, HRP for AEC, Mouse & Rabbit, no chromogen	NB-23-00046-1 / NB-23-00046-2	110ml / 60ml	Simpomount (Water Based, universal) kit	NB-23-00157-1 / NB-23-00157-2	100ml / 18ml
AEC concentrated kit (20x) for 2400 slides	NB-23-00140	12 ml	DAB+ 2 components	NB-23-00148-1	12 ml

**Precautions:**

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

**FOR RESEARCH USE ONLY**