NeoStain ABC Kit, HRP Detection Kit for Mouse and Rabbit Antibodies, with AEC

NB-23-00007

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(Horseradish-peroxidase labeled-streptavidin-biotin detection system for broadspectrum with AEC chromogen)

#Cat: NB-23-00007-1 Size: 18ml, with AEC
#Cat: NB-23-00007-2 Size: 6ml, with AEC

Intended Use:

NeoStain ABC Kit, HRP detection (AEC) kit is intended for using with mouse and rabbit primary antibody (user-supplied) to detect the presence of antigens in human tissue or cell preparations under light microscopy. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells.

Horseradish peroxidase (HRP) labeled-streptavidin and biotinylated secondary antibody amplification system has become a standard technique in immunochemical staining[1,2]. NeoStain ABC Kit, HRP detection (AEC) kit uses human-absorbed, biotinylated, affinity-purified secondary antibody reacts with the user supplied primary antibody bound to the specific epitope of the antigen in tissue or cell. Horseradish peroxidase (HRP) labeled streptavidin then reacts with biotinylated secondary antibody to form a HRP-streptavidin-biotin complex. The HRP enzyme of the streptavidin complex catalyzed the substrate/chomogen, 3-Amino-9ethylcarbazole (AEC substrate) reaction to form red color deposit at the antigen site. The antigen then can be visualized under microscope. Compared to traditional ABC method which uses avidin, streptavidin in NeoStain ABC Kit, HRP detection (AEC) kit demonstrates stronger binding strength to bind biotin and less non-specific background staining. Pre-Block Solution in the kit will help to eliminate non-specific background.

Higher sensitivity and lower background give NeoStain ABC Kit, HRP detection (AEC) kit a higher signal-noise ratio. More than sufficient volume of AEC chormogen is provided in the kit so that customers may use 2 drops of AEC chomogen per ml to obtain higher sensitivity and contrast.

Kit Components:

	1	**2**	**3**	**4A**	**4B**	**4C**
Cat. No.	PreBlocking Solution	Biotinylated second antibody broad spectrum	Streptavidin peroxidase conjugate	Concentrated AEC substrate buffer (20x)	Concentrated AEC chromogen (20x)	Concentrated hydrogen peroxide (20x)
NB-23-00007-2	6ml	6 ml	6 ml	1 ml	2ml	1ml
NB-23-00007-1	18 ml	18 ml	18 ml	2 ml	4 ml	2 ml

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 deparffinized with xylene and rehydrated with a graded series of ethanol before staining.

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4. Cell smear samples should be made as much monolayer as possible to obtain satisfactory results.
5. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slide treated with Isotype control reagent), and negative control.
6. Start staining procedures: DO NOT let specimen or tissue dry from this point on.

Reagent	**Staining Procedures**	**Incubation Time (Min.)**
1. Peroxidase blocking reagent: Supplied by user. We recommend using Peroxidase Block NB-23-00192- 1 / -2.	a. Apply 2 drops (100 µL) or enough volume of Peroxidase blocking reagent (Ready-to-use 3% H2O2 solution) to cover thetissue section and incubate b. Rinse the slide using distilled water.	10 min.
2. HIER Pretreatment: referto antibody spec. sheet	a. Heat Induced Epitope Retrieval (HIER) may be required forprimary antibody suggested by vendor b. Wash with PBS 2 min., 3 times.	
3. **Reagent 1:** Pre-blocking Solution	a. Add 2 drops or enough volume of Pre-blocking Solution tocover the tissue section completely and Incubate b. Blot off solution. DO NOT RINSE.	10 min.
4. Primary antibody: Supplied by user. Investigator needs to optimize dilution and incubation time.	a. Apply 2 drops or enough volume of Primary antibody tocover the tissue section completely. Incubate in moist chamber for 30-60 min. b. Rinse with PBS for 2 min., 3 times.	30-60 min.
5. **Reagent 2:** Ready to use Secondaryantibody	a. Apply 2 drops or enough volume of secondary antibody to coverthe tissue section completely and incubate. b. Rinse with PBS for 2 min., 3 times.	10 min.
6. **Reagent 3:** Ready to use HRPStreptavidin	a. Apply 2 drops or enough volume of HRP-Streptavidin to coverthe tissue section completely and incubate. b. Rinse with PBS for 2 min., 3 times.	10 min.
7. **Reagents 4A, 4B and 4C**:AEC Chromogen	a. Add 1 drop of Reagent 4A, 1 drop or 2 drops (for higher sensitivityand contrast) of Reagent 4B and 1 drop of Reagent 4C to 1 mL distilled or deionized water. Mix well. Protect from light and use within one hour. b. Apply 2 drops (100 µL) or enough volume of pre-mixed AECCHROMOGEN to completely cover tissue and Incubate. c. Rinse with distilled water for 2 min, 3 times.	5-10 min.
8. Hematoxylin**:** Supplied by user	a. Counterstain with 2 drops or enough volume to cover tissuecompletely and wait about 10-20 seconds. b. Rinse thoroughly under tap water for 1-2 min. c. Put slides in PBS until show blue color (about 30-60 seconds) d. Rinse well in distilled water	

9. Mounting media: Supplied by user	**AEC is alcohol soluble, DO NOT dehydrate.** Follow the manufacturedata sheet procedure for mounting. Recommended product: NeoBio Mount AQ: Cat.# NB-23-00155-3 (18ml) NeoBio Mount Universal: Cat.# NB-23-00157-2 (18ml)	

Protocol Notes:

1. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.
2. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining

Precautions:
Handle all specimens as potential infectious materials, wear gloves and protection cloth when handling all reagents.

Remarks:
For research use or investigation only. Not for diagnostic or therapeutic use.

Storage:
Stote at 4°C.

References:

1. Elias, J.M. et al. Sensitivity and Detection Efficiency of the Peroxidase antiperoxidase (PAP) Avidin-Biotin Peroxidase Complex (ABC), and Peroxidase-Labeled Avidin-Biotin (LAB Methods. AM J Clin Pathol 92:62-67, 1989.
2. Polak, J.M and Van Noorden, S. Introduction to Immunocytochemistry Second Edition. Bios Scientific Publishers. 41-54. 1997.

Related Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
NeoStain ABC Kit, HRP, Mouse, no chromogen	NB-23-00003-2	110mL		Simplified HRP RabbitKit (Concentrated, suggested 1:100-200)	NB-23-00010	1 mL
NeoStain ABC Kit, HRP, Mouse, with DAB	NB-23-00003-3 NB-23-00003-4	18 mL 6 mL		Simplified HRP Mouse Kit (Concentrated,suggested 1:100-200)	NB-23-00011	1 mL
NeoStain ABC Kit, HRP, Rabbit, no chromogen	NB-23-00005-2	110mL		Streptavidin-HRP (RTU)	NB-23-00026-2 NB-23-00026-3	18 mL 6 mL
NeoStain ABC Kit, HRP, Rabbit, with DAB	NB-23-00005-3 NB-23-00005-4	18mL 6mL		NeoStain ABC Kit, HRP, Mouse & Rabbit, with AEC	NB-23-00007-1 NB-23-00007-2	18 mL 6 mL
NeoStain ABC Kit, HRP, Goat, no chromogen	NB-23-00012-1	110mL		NeoStain ABC Kit, HRP, Mouse, with AEC	NB-23-00008-1 NB-23-00008-2	18 mL 6 mL
NeoStain ABC Kit, HRP, Goat, with DAB	NB-23-00012-2 NB-23-00012-3	18 mL 6 mL		NeoStain ABC Kit, HRP, Rabbit, with AEC	NB-23-00009-1 NB-23-00009-2	18 mL 6 mL