NeoStain ABC detection Kit HRP, Goat

NB-23-00014

NeoStain ABC Kit, HRP, Goat

(Horseradish peroxidase labeled-streptavidin-biotin detection system for Goat antibody with AEC)

#Cat: NB-23-00014-1 Size: 18 mL
#Cat: NB-23-00014-2 Size: 6 mL

Storage: 2- 8ºC

Intended Use:

NeoStain ABC HRP Goat Detection kit is intended for using with Goat 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. NeoStain ABC HRP Goat AEC Detection kit uses humanabsorbed, 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/chromogen, 3, 3' diaminobenzidine reaction to form brown color deposit at the antigen site. The antigen then can be visualized under microscope. Compared to traditional ABC method which uses avidin, NeoStain ABC HRP Goat AEC Detection kit demonstrates stronger binding strength to bind biotin and less non-specific background staining. Pre-Blocking Solution in the kit will help to eliminate non-specific background. Higher sensitivity and lower background give NeoStain ABC HRP Goat AEC Detection kit a higher signal-noise ratio. More than sufficient volume of AEC chromogen is provided in the kit so that customers may use 2 drops of AEC chromogen per ml to obtain higher sensitivity and contrast.

Kit Components:

	Reagent	1	2	3	4
Catalog No.	NeoStain ABC Kit, HRP, Goat	Pre-Blocking Solution	Biotinylated antiGoat second antibody	Streptavidin peroxidase conjugate	**4A :** AEC substrate (RTU) **4B :** AEC chromogen (Concentrated)
NB-23-00014-2	with AEC	6 ml	6 ml	6 ml	4A: 12ml 4B: 1.5ml
NB-23-00014-1	with AEC	18 ml	18 ml	18 ml	4A: 15ml x 1 4B: 2ml

Neo Biotech
74 rue des Suisses – 92000 Nanterre

Recommended Protocol:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, user needs to supplyappropriately 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 ethanolbefore staining.
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 (slidetreated 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.	a. Apply 2 drops (100 µL) or enough volume of Peroxidase blocking reagent (Ready-to-use 3% H_2O_2 solution) to cover the tissue sectionand incubate b. Rinse the slide using distilled water.	10 min.
2. HIER Pretreatment: refer to antibody spec.sheet	a. Heat Induced Epitope Retrieval (HIER) may be required for primary 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 to completely cover the tissue section and Incubate b. Blot off solution. **DO NOT RINSE**.	10 min.
4. Primary antibody: Supplied by user. Investigator needs tooptimize dilution and incubation time.	a. Apply 2 drops 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
5. Reagent 2: Ready to use Secondary antibody	a. Apply 2 drops or enough volume of secondary antibody to cover thetissue section completely and incubate. b. Rinse with PBS for 2 min., 3 times.	10 min.
6. Reagent 3: Ready to use HRP-Streptavidin	a. Apply 2 drops or enough volume of HRP-Streptavidin to cover the tissue section completely and incubate. b. Rinse with PBS for 2 min., 3 times.	10 min.

7. Reagents 4A, 4B: **4A:** AEC Substrate (RTU) **4B:** AEC Chromogen Concentrate	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of **Reagent4B** to 1ml of 4A. Mix well. Protect from light and use within 5 hours. b. Apply 2 drops (100 µL) or enough volume of pre-mixed AEC chromogen to completely cover tissue and Incubate 5 minutes. c. Rinse with distill water for 2 min, 3 times.	5 min.
8. Hematoxylin: Supplied by user	a. Counterstain with 2 drops (100 ul) or more drops 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	Follow the manufacture data sheet procedure for mounting.Recommended product: NeoMount Perm: Cat. No. **NB-23-00156** (15ml) NeoMount Universal: Cat. No. **NB-23-00157-2** (18ml) or **NB-23-00157-1** (100ml)	

Protocol Notes:

1. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubationtime 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

Precautious:

Handle all specimens as potential infectious materials, wear gloves and protection cloth

FOR RESEARCH USE