NeoStain ABC Kit, HRP, Sheep

Horseradish peroxidase labeled-streptavidin-biotin detection system for Sheep antibody with DAB chromogen

NB-23-00015-1 (60mL; no chromogen)

NB-23-00015-2 (18mL; with DAB)

NB-23-00015-3 (6mL; with DAB)

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NB-23-00015-1; NB-23-00015-2; NB-23-00015-3

Storage: 2-8ºC

INTENDED USE:

The NeoStain ABC Kit, HRP, Sheep, with DAB Kit is intended for use with sheep primary antibodies (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 and paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells.

The Horseradish peroxidase (HRP) labeled-streptavidin and biotinylated secondary antibody amplification system has become a standard technique in immunochemical staining. NeoBiotech's NeoStain ABC, HRP, Sheep, with DAB kit uses human-adsorbed, biotinylated, affinity-purified secondary antibody to detect the user supplied primary antibody bound to specific antigen epitopes in tissue or cell preparations. Horseradish peroxidase (HRP) labeled streptavidin then reacts with biotinylated secondary antibody to form an HRP-streptavidin-biotin complex. The HRP enzyme of the streptavidin complex catalyzes the substrate/chromogen, 3, 3' diaminobenzidine (DAB substrate) to form a brown colored deposit at the antigen site. The antigen can then be visualized under a microscope. When compared to the traditional ABC method which uses avidin, NeoStain ABC, HRP, Sheep, with DAB Kit demonstrates stronger binding strength to biotin with less non-specific background staining.

Higher sensitivity and lower background give our NeoStain ABC, HRP, Sheep, with DAB kit a higher signal-noise ratio. More than sufficient volume of DAB chromogen is provided in the kit so that customers may use 2 drops of DAB chromogen per ml to obtain higher sensitivity and contrast.

KIT COMPONENTS:

Component No.	Content	6mL Kit	18mL Kit	60mL Kit
Reagent 1	Pre-Blocking Solution(RTU)	6mL	18mL	60mL
Reagent 2	Biotinylated anti-Sheep IgG (RTU)	6mL	18mL	60mL
Reagent 3	Streptavidin-HRP (RTU)	6mL	18mL	Not Provided
Reagent 4A	DAB Substrate (RTU)	12mL	15mL x 2	Not Provided
Reagent 4B	DAB chromogen Concentrated (20x)	1.5ml	2mL	Not Provided

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 needs to be adhered to the slide tightly to avoid falling off.
3. Paraffin embedded sections must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made up to as much of a 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.

Step/Reagent	Staining Procedure	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 section and incubate. b. Rinse with 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/ 0.05% Tween20 for 2 min., 3 times.	
3. Reagent 1: Pre-blocking Solution (optional)	This step is optional. Recommend to use rabbit serum as needed. a. Apply 2 drops or enough volume of **Reagent 1** to cover the tissue section completely. Incubate in moist chamber for 10 min. 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 to cover the tissue section completely. Incubate in moist chamber for 30-60 min. b. Rinse with PBS/ 0.05% Tween20 for 2 min., 3 times.	30-60 min
5. Reagent 2: Biotinylated anti-Sheep IgG (RTU)	a. Apply 2 drops or enough volume of **Reagent 2** to cover the tissue section completely. Incubate in moist chamber for 10 min. b. Rinse with PBS/ 0.05% Tween20 for 2 min., 3 times.	10 min.

6. Reagent 3: Streptavidin-HRP (RTU)	a. Apply 2 drops or enough volume of **Reagent 3** to cover the tissue section completely. Incubate in moist chamber for 10 min. b. Rinse with PBS/ 0.05% Tween20 for 2 min., 3 times	10 min.
7. Reagent 4A, 4B: **4A:** DAB Substrate (RTU) **4B:** DAB Chromogen Concentrate(20x)	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of **Reagent 4B** to 1ml of **Reagent 4A**. Mix well. Protect from light and use within 7 hours. b. Apply 2 drops (100µL) or enough volume of pre-mixed DAB. Chromogen to completely cover tissue and Incubate for 5 min. c. Rinse with distilled water for 2 min.	5 min
8. Hematoxylin: Supplied by user	a. Counterstain with 2 or more drops to cover tissue completely and incubate for 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** (18ml) **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 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

RELATED PRODUCTS:

Product	Catalog No	Size		Product	Catalog No	Size
NeoStain ABC Kit, HRP, Mouse & Rabbit, no chromogen	NB-23-00001-3	110 ml		NeoStain ABC Kit, HRP, Mouse & Rabbit, with AEC	NB-23-00007-1 NB-23-00007-2	18ml 6ml
NeoStain ABC Kit, HRP, Mouse & Rabbit, with DAB	NB-23-00001-5 NB-23-00001-6	18 ml 6ml		NeoStain ABC Kit, HRP, Mouse, with AEC	NB-23-00008-1 NB-23-00008-2	18ml 6ml
NeoStain ABC Kit, HRP, Mouse, no chromogen	NB-23-00003-2	110ml		NeoStain ABC Kit, HRP, Rabbit, with AEC	NB-23-00009-1 NB-23-00009-2	18ml 6ml
NeoStain ABC Kit, HRP, Rabbit, with DAB	NB-23-00003-3 NB-23-00003-4	18ml 6ml		Streptavidin-HRP (RTU)	NB-23-00026-2 NB-23-00026-3	110ml 18ml
NeoStain ABC Kit, HRP, Rabbit, no chromogen	NB-23-00005-2	110ml		Simplified HRP Rabbit Kit (Concentrated, suggested 1:100-200)	NB-23-00010	1ml
NeoStain ABC Kit, HRP, Rabbit, with DAB	NB-23-00005-3 NB-23-00005-4	18ml 6ml		Simplified HRP Mouse Kit (Concentrated, suggested 1:100-200)	NB-23-00011	1ml
NeoStain ABC Kit, HRP, Goat, no chromogen	NB-23-00012-1	110ml		NeoStain ABC Kit, HRP, Goat, with DAB	NB-23-00012-2 NB-23-00012-3	18ml 6ml

PRECAUTIONS:

DAB may be carcinogenic. Handle all specimens as potentially infectious materials, wear gloves and appropriate personal protection equipment.

FOR RESEARCH USE ONLY

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