NeoStain ABC Kit, AP, Mouse & Rabbit, no chromogen

NB-23-00023-1
NB-23-00023-2
NB-23-00023-5
NB-23-00023-6

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#Cat: NB-23-00023-1 Size: 110ml
#Cat: NB-23-00023-2 Size: 60ml
#Cat: NB-23-00023-5 Size: 18ml
#Cat: NB-23-00023-6 Size: 6ml

Intended Use:

NeoStain ABC Kit uses biotinylated secondary antibody and Alkaline Phosphatase (AP) labeled-streptavidin to detect mouse and/or rabbit primary antibody (user-supplied) that bind to antigens in human tissue or cell preparations under light microscopy. The most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. Alkaline Phosphatase (AP) labeled-streptavidin and biotinylated secondary antibody amplification system has become a standard technique in immunochemical staining[1,2]. NeoStain ABC 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. Alkaline Phosphatase (AP) labeled streptavidin then reacts with biotinylated secondary antibody to form an AP-streptavidin-biotin complex. The AP enzyme of the streptavidin complex catalyzes the substrate/chomogen such as Fast-Red, Permanent Red, or BCIP/NBT to form a red (Fast-Red or Permanent Red) or dark blue/purple (BCIP/NBT) color deposit at the antigen site. The antigen then can be visualized under microscope. Compared to traditional ABC methods which uses avidin, **NeoStain** ABC Kit demonstrates stronger binding strength to bind biotin and less non-specific background staining. Higher sensitivity and lower background give this kit a higher signal-noise ratio. It also provides users cost effective method for their research. End users may choose Fast-Red, Permanent Red, or BCIP/NBT chromogen depending on their preferences.

Kit Components:

Component No.	Content	NB-23-00023-6	NB-23-00023-5	NB-23-00023-2	NB-23-00023-1
Reagent 1	Pre-Block Solution (RTU)	6mL	18mL	60mL	110mL
Reagent 2	Biotinylated anti-Mouse & Rabbit (RTU)	6mL	18mL	60mL	110mL
Reagent 3	Streptavidin-AP (RTU)	6mL	18mL	60mL	110mL
Reagent 4A	Permanent Red Substrate (RTU)	7mL	18mL	NA	NA
Reagent 4B	Permanent Red Activator (5x)	1.4mL	3.6mL	NA	NA
Reagent 4C	Permanent Red Chromogen (100x)	70µL	180µL	NA	NA

Recommended Protocol:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, the 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 into as thin 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.
7. We recommend TBS-T to be used as the wash buffer to get the highest sensitivity and clean background. Phosphate in the PBS-T may inhibit the activity of the alkaline phosphatase. Note: 1X TBS-T =50mM Tris HCl, 150mM NaCl, 0.05% Tween-20 pH7.6. sells 10xTBS-T for your convenience (NB-23-00201)

Reagent	Staining Procedures	Incubation Time
1.HIER Pretreatment: refer to antibody spec. sheet	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody. Refer to antibody datasheet. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T** (See note 7 above); 3 times for 2 minutes each.	
2. **Reagent 1:** Pre-blocking Solution (RTU)	a. Add 2 drops or enough volume of **Regent 1** (Pre-blocking Solution) to completely cover the tissue section and incubate for 10 min. b. Blot off solution. **DO NOT RINSE**.	1 min.
3. Primary antibody: Supplied by user.	Note: 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 30-60min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	30-60 min.
4. **Reagent 2:** Biotinylated anti-Mouse & Rabbit (RTU)	a. Apply 2 drops or enough volume of **Reagent 2** (Biotinylated anti-Mouse & Rabbit) to cover the tissue section completely and incubate for 10min. b. Wash with PBS-T containing 0.05% Tween-20 or **1X TBS-T**; 3 times for 2 minutes each.	10 min.
5. **Reagent 3:** Streptavidin-AP (RTU)	a. Apply 2 drops or enough volume of **Reagent 3** (Streptavidin-AP) to cover the tissue section completely and incubate for 10 min. b. Wash with **1xTBS-T** only, 3 times for 2 minutes each.	10 min.
6. **Reagent 4:** Chromogen: Fast-Red, or Permanent Red **(To get maximum sensitivity of AP polymer, please repeat chromogen step**), or	Refer to manufacture data sheet if chromogen is supplied by user. Recommended protocol for chromogen using our kit: **1. Fast Red:** a. Dissolve one Fast Red tablet into one 5mL substrate buffer. Vortex until tablet is dissolved. It usually takes 20 minutes to dissolve completely. b. Chromogen must be used within 1 hour.	

BCIP.NBT	c. Apply 100ul or more Fast-Red solution to completely cover the tissue section and incubate 10 minutes at room temperature. d. After proper color development, wash with distill water for 2 minutes, 3 times e. DO NOT Dehydrate tissue after staining. Fast-Red is alcohol soluble. **2. Permanent Red:** **Note**: Shake Permanent Red Activator before adding into Permanent Red Substrate. a. Add 200µL of Reagent 7B (Activator) into 1mL of Reagent 7A (Substrate) and mix well. Add 10µL of Reagent 7C (Chromogen) into the mixture and mix well. [Note: For fewer slides, add 100µL of Reagent 7B (Activator) into 500µL of Reagent 7A (Substrate) and mix well. Add 5µL of Reagent 7C (Chromogen) into the mixture and mix well.] b. Apply 2 drops (100µL) or enough volume of Permanent Red working solution to completely cover the tissue. Incubate for 10 min, observe appropriate color development. **To increase AP signal aspirate or tap off chromogen and apply 2-3 drops (100µL) again of the Permanent Red working solution to completely cover the tissue for additional 5 to 10 min.** c. Rinse well with distilled water. **3. BCIP/NBT** : order separately, Cat. No. NB-23-00144-1or NB-23-00144-2 a. Add two drops (about 100ul) of Ready-to-use BCIP/NBT to cover the tissue section for 5-10 minutes. Monitor the color development under a microscope. b. Rinse with distill water for 2 minutes, 3 times.	
7.Hematoxylin: Supplied by user	a. Counterstain with 2 drops or enough volume to cover tissue completely 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	
8. Mounting media: Supplied by user	Follow the manufacturer's data sheet procedure for mounting. Recommended product: 1. NeoBio Mount AQ: Cat. No. NB-23-00155-3 (18mL) for AEC, Fast-red, Permanent Red and AP-blue, DAB, BCIP/NBT. 2. NeoBio Mount Perm: Cat. No. NB-23-00156(18mL), for DAB and BCIP/NBT 3. NeoBio Mount Universal: Cat.No. NB-23-00157-2 (18mL), or NB-23-00157-1 (100mL), universal permanent mounting medium	

Protocol Notes:

1. The fixation, tissue slide thickness, antigen retrieval and primary antibody dilution and incubation time affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting 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 Prouducts:

Product	Catalog No.	Size
NeoStain ABC Kit, AP, Mouse, no chromogen	NB-23-00024-1	110mL
NeoStain ABC Kit, AP, Mouse, with Permanent Red	NB-23-00024-4 / NB-23-00024-5	18mL / 6mL
NeoStain ABC Kit, AP, Rabbit, no chromogen	NB-23-00025-1	110mL
NeoStain ABC Kit, AP, Rabbit, with Permanent Red	NB-23-00025-4/ NB-23-00025-5	18mL / 6mL
Streptavidin-AP (RTU)	NB-23-00027-1/ NB-23-00027-2	100mL / 18mL
AP-Red concentrated (40x) Kit for 3000 slides	NB-23-00143	8mL
BCIP/NBT (RTU)	NB-23-00144-1/ NB-23-00144-2	100mL / 18mL
NeoBio Mount AQ (Aqueous solution, use with AEC and AP-Red)	NB-23-00155-3	18mL
NeoBio Mount Perm (Permanent mount for DAB,BCIP/NBT)	NB-23-00156	18mL
NeoBio Mount Universal (Water Based, universal) Kit	NB-23-00157-1/ NB-23-00157-2	100mL / 18mL

Precautions:

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

Remarks:

For research use only.

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.