Polymer Extra Anti-Hamster-Armenian HRP-DAB Kit

ORDER INFORMATION

Name of Products: Polymer Extra Anti Hamster-Armenian HRP-DAB Kit
Catalogue Number: **nAP-10080-3**
Size: **11 ml kit (w DAB chromogen and buffer) for 100 slides**
Storage: **4-8°C**

Intended Use:

Polymer Extra anti Hamster-Armenian HRP-DAB kit is the 3rd generation of polymer detection system. It uses Hamster-Armenian antibody specific enhancer to help amplify the polymer-enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of Polymer Extra detection system. It is a biotin-free system, therefore, overcomes the non-specific staining caused by endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. It can be used for manual stain or autostainer. Staining conditions need to be optimized by user. AP's Polymer Extra HRP Detection System offers a wide choice of primary antibodies, including broad spectrum (for mouse and rabbit primary antibodies), rabbit, mouse, goat, sheep, and rat primary antibodies. Refer to Related Product section for details.

Kit components:

Components	Name	Volume
Reagent 1	Hamster-Armenian Antibody Enhancer (Ready To Use)	**11ml**
Reagent 2	Polymer HRP for anti-Hamster-Armenian IgG (Ready To Use)	**11ml**
Reagent 3A	DAB Substrate (Ready To Use)	**20ml**
Reagent 3B	DAB Chromogen (20x)	**1ml**

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 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.

Reagent	Staining Procedure	Incubation Time
1. PEROXIDASE BLOCKING REAGENT. Supplied by user	a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% H2O2 solution) for 10 minutes. b. Rinse the slide using distilled water.	10min
2. HIER PRETREATMENT:	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.	
3. PRE-BLOCK (Optional) Not provided	a. Add 2 drops (100µL) or enough volume of Protein Block Buffer (nAP-20003) to completely cover the tissue section, Incubate for 10 min. b. Drain or blot off solution. DO NOT RINSE c. See note 8 in Recommended Protocol.	10min
4. PRIMARY ANTIBODY Supplied by user	a. Apply 2 drops (100µL) 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.	30-60min
5. Reagent 1 Hamster-Armenian antibody enhancer (RTU)	a. Apply 2 drops (100µL) or enough volume of Reagent 1 Hamster-Armenian antibody enhancer to cover each section. Incubate in moist chamber for 10-30min (**for 30min if stronger signal is needed**). b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	10-30min
6. Reagent 2 Polymer HRP anti-Hamster-Armenian IgG (RTU)	a. Apply 2 drops (100µL) or enough volume of Reagent 2 Polymer HRP anti- Hamster-Armenian IgG to cover each section. Incubate in moist chamber for 15-30 min (**for 30min if stronger signal is needed**).	10-30min
	b. Wash with PBS-T (PBS containing 0.05% Tween-20) 3 times for 2 minutes each time.	
7. CHROMOGEN Reagent 3A: DAB Substrate(RTU) Reagent 3B: DAB Chromogen (20x)	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.	5min
8. HEMATOXYLIN Supplied by user	a. Counterstain with 2 drops (100µL) or enough volume of Hematoxylin to cover tissue completely and wait about 20-30 seconds. 20 seconds b. Rinse well with running tap water for 1-2 minutes. c. Put slides in PBS until show blue color (about 30-60 seconds). d. Rinse well in distill or tap water	20 Second
9. Mounting Medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: Angio-Proteomie: Cat. No. nAP-20002	Refer to data sheet

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

Remarks:
For research use only.

Caution: Handling human tissue derived products is potentially bio-hazardous, always wear gloves and safety glasses when working on these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.