APlink AP Broad Detection Kit for Mouse and Rabbit Antibodies

(Alkaline Phosphatase labeled streptavidin-biotin detection system for broad spectrum)

Storage: 4-8ºC

Catalog No.:	D07-110	110ml	☐
	D07-60	60ml	☐
	D07-18F	18ml	☐
	D07-6F	6ml	☐
	D07-18A	18ml	☐
	D07-6A	6ml	☐

Intended Use:

APlink AP Broad Detection 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]. APlink AP Broad Detection Kit uses human-absorbed, bioinylated, 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, AP-Red, or BCIP/NBT to form a red (Fast-Red or AP-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, APlink AP Broad Detection 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, AP-Red, or BCIP/NBT chromogen depending on their preferences.

Kit Components:

		Reagent 1	Reagent 2	Reagent 3	Reagent 4
Catalog No.	**Description**	Pre-Blocking Solution	Biotinylated second antibody broad spectrum	Streptavidin-AP conjugate	Chromogen
D07-110	APlink AP Broad Bulk Kit	110 ml	110 ml	110 ml	Not included
D07-60	APlink AP Broad Bulk Kit	60ml	60ml	60ml	Not included
D07-18F	APlink AP Broad Fast Red Kit	18ml	18ml	18ml	a. 15 Fast Red tablets b. 80ml Substrate buffer (RTU)
D07-6F	APlink AP Broad Fast Red Kit	6ml	6ml	6ml	a. 6 Fast Red tablets b. 35ml Substrate buffer (RTU)
D07-18A	APlink AP Broad AP-Red+ Kit	18ml	18ml	18ml	a. 1.5ml AP-Red+ Enhancer (40x) b. 1.5ml AP-Red+ Solution (40x) c. 6ml AP-Red+ Substrate (20x)
D07-6A	APlink AP Broad AP-Red+ Kit	6ml	6ml	6ml	a. 1ml AP-Red+ Enhancer (40x) b. 1ml AP-Red+ Solution (40x) c. 4ml AP-Red+ Substrate (20x)

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 deparffinized 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.

9700 Harbour Place, Suite 129, Mukilteo, WA 98275, USA Tel: 425.493.1801 Fax: 425.493.1803 http://www.gbi-inc.com

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
1. 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 3 times for 2 minutes each time.	
2. **Reagent 1:** Pre-blocking Solution	a. Add 2 drops or enough of volume Pre-blocking Solution to completely cover the tissue section and Incubate for 10 min. b. Blot off solution. **DO NOT RINSE**.	10 min.
3. 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 3 times for 2 minutes each time.	30-60 min.
4. **Reagent 2:** Ready-to-use Broad Secondary antibody	a. Apply 2 drops or enough volume of secondary antibody to cover the tissue section completely and incubate for 10 min. b. Rinse with PBS 3 times for 2 minutes each time.	10 min.
5. **Reagent 3:** Ready-to-use AP-Streptavidin	a. Apply 2 drops or enough volume of AP-Streptavidin to cover the tissue section completely and incubate for 10 min. b. Rinse with PBS 3 times for 2 minutes each time. c. Rinse with tap water.	10 min.
6. **Reagent 4:** Chromogen: Fast-Red, or AP-red, or BCIP.NBT	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. 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. <u>DO NOT Dehydrate tissue after staining. Fast-Red is alcohol soluble.</u>** **2. AP-Red+ (40x):** a. Add 1 drop (50ul) of AP-Red+ Enhancer and 1 drop (50ul) of AP-Red+ Solution to a test tube. Mix well and set at room temperature for about 5 minutes. b. Add 2ml of distilled water to the mixture. Mix well. c. Add 4 drops (200ul) of AP-Red+ Substrate to the mixture and mix well. d. Completely cover the tissue section with the mixture and incubate for 5-15 minutes. e. After proper color development, wash with distill water 2 minutes, 3 times**. AP-Red+ is soluble in organic solvent. Do not dehydrate**. **3. BCIP/NBT :** order separately, Cat. No. C05-100 or C05-18 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. GB-Mount: Cat. No. E01-18 (18ml) for AEC, Fast-red, AP-Red and AP-blue, DAB, BCIP/NBT. 2. O-Mount: Cat. No. E02-18 (18ml), for DAB and BCIP/NBT 3. Simpo-Mount: Cat.No. E03-18 (18ml), or E03-100 (100ml), universal permanent	

9700 Harbour Place, Suite 129, Mukilteo, WA 98275, USA Tel: 425.493.1801 Fax: 425.493.1803 http://www.gbi-inc.com

	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 Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
APlink AP Mouse Bulk kit	D08-110	110ml		Fast Red Kit	C03-60	12 Tab + 60ml
APlink AP Mouse Fast Red Kit	D08-18F / D08-6F	18ml / 6ml		AP-Red+ Kit (40x concentrate)	C04-8	8ml
APlink AP Mouse AP-Red+ Kit	D08-18A / D08-6A	18ml / 6ml		BCIP/NBT Kit	C05-100 / C05-18	100ml / 18ml
APlink AP Rabbit Bulk Kit	D09-110	110ml		GB-Mount (Aqueous)	E01-18	18ml
APlink AP Rabbit Fast Red Kit	D09-18F / D09-6F	18ml / 6ml		O-Mount (Organic)	E02-18	18ml
APlink AP Rabbit AP-Red+ Kit	D09-18A / D09-6A	18ml / 6ml		Simpo-Mount (Aqueous)	E03-100 / E03-18	100ml / 18ml
Streptavidin-AP (RTU)	D29-110 / D29-18	100ml / 18ml				

Precautious:

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.