

## SPLink HRP Detection (AEC) Kit for Mouse and Rabbit Antibodies

(Horseradish-peroxidase labeled-streptavidin-biotin detection system for broad spectrum with AEC chromogen)

Storage: 4-8°C

Catalog No.: D04-6      6 ml   

D04-18      18 ml   

### Intended Use:

SPLink HRP Broad detection (AEC) kit is intended for using with mouse and rabbit 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<sup>1,2</sup>. SPLink HRP Broad detection (AEC) 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. 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/chomogen, 3-Amino-9-ethylcarbazole (AEC substrate) reaction to form red color deposit at the antigen site. The antigen then can be visualized under microscope. Compared to traditional ABC method which uses avidin, streptavidin in SPLink HRP Broad detection (AEC) kit demonstrates stronger binding strength to bind biotin and less non-specific background staining. Pre-Block Solution in the kit will help to eliminate non-specific background.

Higher sensitivity and lower background give SPLink HRP Broad detection (AEC) 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:

Cat. No.	No. Description	1	2	3	4A	4B	4C
		Pre-Blocking Solution	Biotinylated second antibody broad spectrum	Streptavidin-peroxidase conjugate	Concentrated AEC substrate buffer (20x)	Concentrated AEC chromogen (20x)	Concentrated hydrogen peroxide (20x)
D04-6	SPLink HRP Broad AEC 6ml Kit	6ml	6 ml	6 ml	1 ml	2ml	1ml
D04-18	SPLink HRP Broad AEC 18ml Kit	18 ml	18 ml	18 ml	2 ml	4 ml	2 ml

### 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 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 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 (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 (Min.)
1. Peroxidase blocking reagent: Supplied by user.	a. Apply 2 drops (100 $\mu$ L) or enough volume of Peroxidase blocking reagent (Ready-to-use 3% H <sub>2</sub> O <sub>2</sub> solution) to cover the tissue section and 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. <b>Reagent 1:</b> Pre-blocking Solution	a. Add 2 drops or enough volume of Pre-blocking Solution to cover the tissue section completely and Incubate 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 for 2 min., 3 times.	30-60 min.
5. <b>Reagent 2:</b> Ready to use Secondary antibody	a. Apply 2 drops or enough volume of secondary antibody to cover the tissue section completely and incubate. b. Rinse with PBS for 2 min., 3 times.	10 min.
6. <b>Reagent 3:</b> 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. <b>Reagents 4A, 4B and 4C:</b> AEC Chromogen	a. Add 1 drop of Reagent 4A, 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 4B and 1 drop of Reagent 4C to 1 mL distilled or deionized water. Mix well. Protect from light and use within one hour. b. Apply 2 drops (100 $\mu$ L) or enough volume of pre-mixed AEC CHROMOGEN to completely cover tissue and Incubate. c. Rinse with distilled water for 2 min, 3 times.	5-10 min.
8. 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	
9. Mounting media: Supplied by user	<b>AEC is alcohol soluble, DO NOT dehydrate.</b> Follow the manufacture data sheet procedure for mounting. Recommended product: GB-Mount: Cat. No. E01-15 (15ml) Simpo-Mount: Cat.No. E03-15 (15ml)	

#### 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
SPlink HRP Mouse Bulk kit	D02-110	110ml		Simplified Streptavidin HRP Rabbit concentrate kit (1:100)	D30-1	1ml
SPlink HRP Mouse DAB Kit	D02-18 / D02-6	18ml / 6ml		Simplified Streptavidin HRP Mouse concentrate kit (1:100)	D31-1	1ml
SPlink HRP Rabbit Bulk kit	D03-110	110ml		SPlink HRP Broad Bulk	D01-110	110ml
SPlink HRP Rabbit DAB Kit	D03-18 / D03-6	18ml / 6ml		SPlink HRP Broad DAB	D01-18 / D01-6	18ml / 6ml
SPlink HRP Goat Bulk kit	D76-110	110ml		SPlink HRP Mouse AEC	D05-18 / D05-6	18ml / 6ml
SPlink HRP Goat DAB Kit	D76-18 / D76-6	18ml / 6ml		SPlink HRP Rabbit AEC	D06-18 / D06-6	18ml / 6ml

**Precautions:**

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

**Remarks:**

For research use or investigation only. Not for diagnostic or therapeutic use.

**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.