

## SPlink HRP Goat Detection (AEC) Kit

(Horseradish peroxidase labeled-streptavidin-biotin detection system for Goat antibody with AEC chromogen)

Storage: 2-8°C

Catalog No.: D77-110 (w/o AEC) 110ml   
 D77-18 18 ml   
 D77-6 6 ml

**Intended Use:**

**SPlink HRP Goat AEC Detection Kit** is intended for using with Goat 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 Goat AEC Detection 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 AEC substrate/chromogen to form red color deposit at the antigen site. The antigen then can be visualized under microscope. When compared to traditional ABC method which uses avidin, **SPlink HRP Goat AEC Detection Kit** demonstrates stronger binding strength to bind biotin and less non-specific background staining. Pre-Blocking Solution in the kit will help to eliminate non-specific background. Higher sensitivity and lower background give our **SPlink HRP Goat AEC Detection Kit** a higher signal-noise ratio.

**Kit Components:**

Component No.	Content	6mL Kit	18mL Kit	110mL Kit
<b>Reagent 1</b>	Pre-Blocking Solution(RTU)	6mL	18mL	110mL
<b>Reagent 2</b>	Biotinylated anti-Goat IgG (RTU)	6mL	18mL	110mL
<b>Reagent 3</b>	Streptavidin-HRP (RTU)	6mL	18mL	110mL
<b>Reagent 4A</b>	AEC Substrate Buffer (20X)	1mL	2mL	Not Provided
<b>Reagent 4B</b>	AEC Chromogen (20X)	2mL	4mL	Not Provided
<b>Reagent 4C</b>	Hydrogen peroxide (20X)	1mL	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 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 µL) 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 (RTU)	a. Add 2 drops or enough volume of Pre-blocking Solution to completely cover the tissue section 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>Reagent 4A, 4B &amp; 4C</b> Reagent 4A: AEC Substrate (20x) Reagent 4B: AEC Chromogen (20x) Reagent 4C: Hydrogen Peroxide (20x)	a. Add 1 drop of <b>Reagent 4A</b> into 1ml distilled water, mix well. Then add 1 drop or 2 drops (for higher sensitivity and contrast) of <b>Reagent 4B</b> and 1 drop of <b>Reagent 4C</b> in the diluted substrate buffer. Mix well. Protect from light and use within 1 hour. b. Apply 2 drops (100 µL) or enough of mixture completely cover tissue. Incubate for about 10 min. Monitor the color development under microscope. c. Rinse with tap water for 1-2 min. <b>AEC is alcohol soluble, do not dehydrate!</b>	10 min.
8. Hematoxylin: Supplied by user	a. Counterstain with 2 drops (100 ul) or more drops 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	Follow the manufacture data sheet procedure for mounting. Recommended product: GB-Mount: Cat. No. E01-18 (18ml) or E01-100 (100ml) Simpo-Mount: Cat. No. E03-18 (18ml) or E03-100 (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

**Precautions:**

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

**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.