SPlink HRP Goat Detection (DAB) Kit

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

Storage: 2-8ºC

Catalog No.: D76-110 (w/o DAB) 110ml ☐
D76 (2)-18 18 ml ☒
D76-18 18 ml ☐
D76-6 6 ml ☐

Intended Use:
SPlink HRP Goat DAB 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[1,2]. SPlink HRP Goat DAB 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 substrate/chomogen, 3,3' diaminobenzidine (DAB substrate) reaction to form brown color deposit at the antigen site. The antigen then can be visualized under microscope. Compared to traditional ABC method which uses avidin, SPlink HRP Goat DAB 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 SPlink HRP Goat DAB Detection kit a higher signal-noise ratio. More than sufficient volume of DAB chormogen is provided in the kit so that customers may use 2 drops of DAB chromogen per ml to obtain higher sensitivity and contrast.

Kit Components:

	Reagent number:	**1**	**2**	**3**	**4A**	**4B**
Catalog No	Name	Pre-Blocking Solution	Biotinylated anti-Goat second antibody	Streptavidin-peroxidase conjugate	DAB substrate (Ready-to-use)	DAB chromogen (Concentrated)
D76-6	SPlink HRP Goat DAB 6ml kit	6ml	6 ml	6 ml	12 ml	1.5ml
D76-18	SPlink HRP Goat DAB 18ml kit	18 ml	18 ml	18 ml	15ml x 2	2 ml
D76 (2)-18	SPlink HRP Goat DAB 18ml kit	Not included	18 ml	Not included	Not included	Not included
D76-110	SPlink HRP Goat Bulk kit	110ml	110ml	110ml	Not included	Not included

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 deparffinized 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.)**

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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_2O_2 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. **Reagent 1:** Pre-blocking Solution	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. **Reagent 2:** 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. **Reagent 3:** 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. **Reagents 4A, 4B:** **4A**: DAB Substrate (RTU) **4B:** DAB Chromogen Concentrate	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent **4B** to 1ml of **4A**. Mix well. Protect from light and use within 5 hours. b. Apply 2 drops (100 µL) or enough volume of pre-mixed DAB chromogen to completely cover tissue and Incubate 5 minutes. c. Rinse with distill water for 2 min, 3 times.	5 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 distiled water	
9. Mounting media: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: O-Mount: Cat. No. E02-18 (18ml) 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

Precautious:

DAB may be carcinogenic. Handle all specimens as potential infectious materials, wear gloves and protection cloth.

Remarks:

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

References:

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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.

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