

SPlink HRP Sheep with DAB Kit

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

Storage: 2-8°C

Catalog No.:	D90-60	60 ml	<input type="checkbox"/>
	D90-18	18 ml	<input type="checkbox"/>
	D90-6	6 ml	<input type="checkbox"/>

Intended Use:

The **SPlink HRP Sheep with DAB Kit** is intended for use with sheep primary antibodies (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 and paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells.

The Horseradish peroxidase (HRP) labeled-streptavidin and biotinylated secondary antibody amplification system has become a standard technique in immunochemical staining^{1,2}. **GBI SPlink HRP Sheep with DAB Kit** uses human-adsorbed, biotinylated, affinity-purified secondary antibody to detect the user supplied primary antibody bound to specific antigen epitopes in tissue or cell preparations. Horseradish peroxidase (HRP) labeled streptavidin then reacts with biotinylated secondary antibody to form an HRP-streptavidin-biotin complex. The HRP enzyme of the streptavidin complex catalyzes the substrate/chomogen, 3,3' diaminobenzidine (DAB substrate) to form a brown colored deposit at the antigen site. The antigen can then be visualized under a microscope. When compared to the traditional ABC method which uses avidin, **GBI SPlink HRP Sheep with DAB Kit** demonstrates stronger binding strength to biotin with less non-specific background staining.

Higher sensitivity and lower background give our **SPlink HRP Sheep with DAB 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:

Component No.	Content	6mL Kit	18mL Kit	60mL Kit
Reagent 1	Pre-Blocking Solution(RTU)	6mL	18mL	60mL
Reagent 2	Biotinylated anti-Sheep IgG (RTU)	6mL	18mL	60mL
Reagent 3	Streptavidin-HRP (RTU)	6mL	18mL	60mL
Reagent 4A	DAB Substrate (RTU)	12mL	15mLx2	60mL
Reagent 4B	DAB chromogen Concentrated (20x)	1.5mL	2mL	3mL

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 falling off.
3. Paraffin embedded sections must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made up to as much of a 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.

Step/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 ₂ O ₂ solution) to cover the tissue section and incubate. b. Rinse with 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/ 0.05% Tween20 for 2 min., 3 times.	
3. Reagent 1: Pre-blocking Solution (optional)	This step is optional. Recommend to use rabbit serum as needed. a. Apply 2 drops or enough volume of Reagent 1 to cover the tissue section completely. Incubate in moist chamber for 10 min. 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/ 0.05% Tween20 for 2 min., 3 times.	30-60 min.
5. Reagent 2: Biotinylated anti-Sheep IgG (RTU)	a. Apply 2 drops or enough volume of Reagent 2 to cover the tissue section completely. Incubate in moist chamber for 10 min. b. Rinse with PBS/ 0.05% Tween20 for 2 min., 3 times.	10 min.
6. Reagent 3: Streptavidin-HRP (RTU)	a. Apply 2 drops or enough volume of Reagent 3 to cover the tissue section completely. Incubate in moist chamber for 10 min. b. Rinse with PBS/ 0.05% Tween20 for 2 min., 3 times.	10 min.
7. Reagent 4A, 4B: 4A: DAB Substrate (RTU) 4B: DAB Chromogen Concentrate(20x)	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 4B to 1ml of Reagent 4A . Mix well. Protect from light and use within 7 hours. b. Apply 2 drops (100µL) or enough volume of pre-mixed DAB. chromogen to completely cover tissue and Incubate for 5 min. c. Rinse with distilled water for 2 min.	5 min.
8. Hematoxylin: Supplied by user	a. Counterstain with 2 or more drops to cover tissue completely and incubate for 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: 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

Related Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
SPlink HRP Broad Bulk kit	D01-110	110ml		SPlink HRP Broad AEC	D04-18 / D04-6	18ml / 6ml
SPlink HRP Broad DAB Kit	D01-18 / D01-6	18ml / 6ml		SPlink HRP Mouse AEC	D05-18 / D05-6	18ml / 6ml
SPlink HRP Mouse Bulk kit	D02-110	110ml		SPlink HRP Rabbit AEC	D06-18 / D06-6	18ml / 6ml
SPlink HRP Mouse DAB Kit	D02-18 / D03-6	18ml / 6ml		Streptavidin Peroxidase (RTU)	D25-110 / D25-18	110ml 18ml
SPlink HRP Rabbit Bulk kit	D03-110	110ml		Simplified Streptavidin HRP Rabbit concentrate kit (1:100)	D30-1	1ml
SPlink HRP Rabbit DAB Kit	D03-18 / D03-6	18ml / 6ml		Simplified Streptavidin HRP Mouse concentrate kit (1:100)	D31-1	1ml
SPlink HRP Goat Bulk kit	D76-110	110ml				
SPlink HRP Goat DAB Kit	D76-18 / D76-6	18ml / 6ml				

Precautions:

DAB may be carcinogenic. 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.