GBI Labs
GOLDEN BRIDGE INTERNATIONAL

コスモ・バイオ株式会社

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SPlink HRP Detection Bulk Kit for Mouse and Rabbit Antibodies

(Horseradish peroxidase labeled streptavidin-biotin detection system for broad spectrum without chromogen)

Storage: 2-8ºC

Catalog No.:		
	D01-110	110ml ☐
	D01-60	60ml ☐
	D01-18	18ml ☐
	D01-6	6ml ☐

Intended Use:

SPlink HRP Detection broad spectrum 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[1,2]. SPlink HRP Broad 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, 3,3ôdiaminobenzidine (DAB substrate) or 3-Amino-9-ethylcarbazole (AEC substrate) reaction to form brown (if use DAB) or red color (if use AEC) deposit at the antigen site. The antigen then can be visualized under microscope. Compared to traditional ABC method which uses avidin, SPlink HRP Broad Detection Bulk kit demonstrates stronger binding strength to bind biotin and less non-specific background staining.

Higher sensitivity and lower background give SPlink HRP Broad Detection Bulk kit a higher signal-noise ratio. SPlink HRP Detection Bulk kit provides users cost effective method for their research. End users may use DAB (Cat. No. C02-12) or DAB+ kit (C09-12) or AEC (Cat. No. C01-12) chromogen.

Kit Components:

	No.	Reagent 1	Reagent 2	Reagent 3	Reagent 4A, B
Cat. No.	**Description**	Pre-Blocking Solution	Biotinylated second antibody broad spectrum	Streptavidin-peroxidase conjugate	4A: DAB Substrate 4B: DAB Chromogen
D01-110	SPlink HRP Broad Bulk Kit	110 ml	110 ml	110 ml	Not included
D01-60	SPlink HRP Broad 60ml Kit	60ml	60ml	60ml	Not included
D01-18	SPlink HRP Broad DAB 18ml Kit	18ml	18ml	18ml	4A: 15ml x2 4B: 2ml
D01-6	SPlink HRP Broad DAB 6ml Kit	6ml	6ml	6ml	4A: 12ml 4B: 1.5ml

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.

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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 ε L) 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 of volume 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. **Reagent 4:** **4A:** DAB Substrate **4B:** DAB Chromogen concentrate (chromogen may be supplied by user)	a. Adding 1 drop or 2 drops (for higher sensitivity and contrast) of DAB chromogen concentrate (Reagent **4B**) in 1ml of DAB substrate buffer (Reagent **4A**). Mix well. b. Apply 2 drops (100 ε L) or enough volume of pre-mixed DAB Chromogen to completely cover tissue. Incubate for 5 min. Use the prepared DAB solution within 5 hours. c. When appropriate color is developed, rinse under tap water gently for about 1-2 minutes.	5 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	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. GB-Mount: Cat. No. E01-15 (15ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. O-Mount: Cat. No. E02-15 (15ml), for DAB 3. Simpo-Mount: Cat.No. E03-15 (15ml), or E03-100 (100ml), universal permanent mounting medium	

Protocol Notes:

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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 day 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		Streptavidin Peroxidase (RTU)	D25-110 / D25-18	110ml 18ml
SPlink HRP Rabbit DAB Kit	D03-18 / D03-6	18ml / 6ml		SPlink HRP Broad AEC	D04-18 / D04-6	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

Precautious:

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:

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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