GBI Simplified Streptavidin-HRP Detection System for Mouse or Rabbit Antibodies

(Concentrated Horseradish-peroxidase labeled-streptavidin-biotin detection system
for mouse OR rabbit antibodies for immuno-histological staining)
Each kit good for 1000-2000 slides

Storage: 2-8ºC

Catalog No.: D30-1(**Rabbit**) 1 ml ☐

D31-1(**Mouse**) 1 ml ☐

Intended Use:

GBI's **Simplified Streptavidin HRP kit** is intended for using with primary antibody reagent (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. Used for research or investigation purpose.

Background:

Simplified Streptavidin HRP kit uses horseradish peroxidase (HRP) labeled streptavidin and biotinylated affinity-purified secondary antibody to create a Streptavidin-Biotin amplification system. Streptavidin, a protein isolated from *Streptomyces Avidinii*, can bind to biotin as egg white avidin. However streptavidin exhibits less non-specific binding in certain tissue specimen compared to avidin. This is because streptavidin has a bioelectric point of 6.5 and avidin has 10. Streptavidin will have a lower charge under certain physiological circumstances, therefore, show lower non-specific binding than avidin. In addition to lower background, streptavidin-biotin also demonstrates superior sensitivity to that of the Avidin-Biotin Complex (ABC) system. The binds between streptavidin and biotin are unusual strong ($K_d=10^{-15}$ M). Higher sensitivity and lower background give streptavidin-biotin amplification system a higher signal-noise ratio.

Principle:

The principle of **Simplified Streptavidin HRP kit** is as follows:

1. Paraffin embedded tissue must be deparaffinized and rehydrated. Treating tissue sections with 3% hydrogen peroxide in absolute methanol to suppress endogenous peroxidase activity
2. Incubating the tissue section with non-immune normal serum to eliminate non-specific binding caused by immunoglobulin cross-reactivity (not provided).
3. Incubate primary antibody with the tissue section. Refer supplier's data sheet for incubation conditions for each antibody. Any excess antibody is removed by washing.
4. Biotinylated secondary antibody (Reagent A) is then added on the tissue section to bind the primary antibody. Unbound antibody is removed by washing.
5. Next, the sample is incubated with the streptavidin-peroxidase (Reagent B) to bind the biotin residue on the secondary antibody. Unbound enzyme is removed by washing
6. After step 5 the HRP-streptavidin-biotin-antibody complex will form and reacts with the primary antibody bound to the specific epitope of the target antigen in the sample.
7. The HRP enzymes then catalyzed the substrate/chromogen (not provided) reaction to form a colored insoluble precipitate (brown for DAB or red for AEC) which demonstrates the location of the antigen in the sample.

Kit Components:

Cat. No.	Reagent A	Reagent B
D30-1	Concentrated Biotinylated second antibody for **rabbit** 1ml	Concentrated Streptavidin-peroxidase conjugate 1ml
D31-1	Concentrated Biotinylated second antibody for **mouse** 1ml	Concentrated Streptavidin-peroxidase conjugate 1ml

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Materials Needed but not Provided:

Xylene, ethanol, and absolute methanol
Distilled or deionized water
30% Hydrogen peroxide
10mM phosphate-buffered saline, pH 7.5 (PBS)
Primary antibody
10% normal goat serum in PBS
1% BSA in PBS
Chromogen/substrate solution (AEC or DAB)
Counterstain solution (Hematoxylin)
Mounting Media

Stability and Storage:

When stored at 2 – 8℃, the GBI Simplified Streptavidin HRP kit is stable up to the expiration date indicated on the label. Do not freeze or expose to elevated or volatile temperature. Do not store kit component or perform staining in strong light, such as direct sun light. Do not use expired reagent.

Precautions:

1. Do not mix reagents from different lot.
2. Do not allow the slides to day at any time during staining.
3. Handle all specimens as potential infectious materials, wear gloves and protection cloth.
4. Do not mouth pipette reagents.
5. Diaminobenzidine (DAB) may be carcinogenic. This solution may cause irritation upon skin contact. Wear gloves when handling DAB. If skin contact occurs, flush affected area with large amount clean water. Dispose must follow local regulation.
6. Since there is a potential hazard of explosion due to the reaction of sodium azide with copper and metal in the plumbing system, flush the drain thoroughly with water after disposal of reagents.

Suggested Staining Protocol:

1. SAMPLE PREPARATION: Specimens should be from fresh and fixed as soon as possible. Appropriate tissue and antigen fixation is essential to obtain reproducible results and reliable interpretations. Fixation methods for the antigen been investigated may be obtained from literatures. Some commonly used fixatives are listed as follows:

1) 10% neutral buffered formalin, B5, Bouin's, Zinc formalin or alcohol-base fixatives are considered as suitable fixatives for most antigens.
2) Before paraffin embedding formalin-fixed tissues post-fixed in B5 may exhibit improved stain.
3) If prepare cell smears sample from body fluids one must assure a monolayer of cells. Multi-layers of cells may impact on staining contact, therefore, interfere the interpretation of the results. Cell smears are stable for one to two weeks when stored at 4℃. However, it is strongly recommended to fix the smear sample immediately after preparation.
4) For cytospin or frozen section fixation can be done with acetone (100%) at 4℃ for 10 minutes.

2. TISSUE SECTION:

1) Pre-coat slides with commercial supplied adhesive reagent. Another option is pre-coat slides with 0.1% poly-L-lysine in water, then dry.
2) Section the tissue approximately four microns thick.
3) Place the sections on the slides as flat and wrinkle free as possible to optimize stain.

3. DEPARAFFINIZATION AND REHYDRATION:

Deparaffinize paraffin sections with xylene, followed by rehydration in a graded series of ethanol. Cell smears or tissue must be washed in a PBS bath for 10 minutes prior to staining.

Note:
Tissue sections should be used the same day they are deparaffinized.

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Do not let specimen or tissue sections dry from this point on.

4. STAINING PROCEDURE: (Do all steps at room temperature)

1) **Quenching endogenous peroxidase activity:** add 1 part of 30% hydrogen peroxide to 9 parts of absolute methanol and mix. Dip the paraffin embedded section in to the mixed reagent for 10 minutes. Rinse well with PBS (2 or 3 min., 3 times).

2) **Blocking:** add 2 drops of Blocking Solution (10% NGS, not provided) to each section. Incubate 10 minutes. Drain or blot off the solution. DO NOT RINSE. This step can be skipped if primary antibody is used with 10% non-immune serum.

3) **Primary antibody:** apply 100 ul (about 2 drops) to each section. The section must be covered by reagent completely. Incubate in moist chamber for 30 – 60 minutes. Optimal dilution and incubation time should be determined by the investigator. Dilution and incubation time will depend on sample preparation, antibody affinity, amount of antigen present, and antigen accessibility. Rinse well with PBS (2 or 3 min., 3 times).

4) **Secondary antibody (Dilute reagent A, 1:100-200 in 1% BSA-PBS solution):** apply 100 ul (about 2 drops) of diluted Biotinylated Secondary Antibody (Reagent A) to each section. Reagent must cover the section completely. Incubate in moist chamber for 10 minutes. Rinse well with PBS (2 or 3 min., 3 times).

5) **Enzyme Conjugate (Diluent reagent B, 1:100-200 in PBS. Do not use Sodium Azide as a preservative):** apply 100 ul (about 2 drops) of diluted Enzyme Conjugate (Reagent B) to each section. Reagent must cover the section completely. Incubate in moist chamber for 10 minutes. Rinse well with PBS (2 or 3 min., 3 times).

6) **Substrate/Chromogen Mixture (not provided)**: Apply 2 drops or 100 µl of Substrate/Chromogen Mixture to each section. Incubate for 3 -5 minutes for DAB and 5 – 10 minutes for AEC. Rinse well with distilled water or running tap water for about 2 minutes.

7) **Hematoxylin (not provided)**: Counterstain the slides with Hematoxylin. Wash slides in tap water. Put slides in PBS until blue (about 30 second). Rinse in distilled water.

8) **Mounting Solution (not provided)**: For AEC kit: apply 2 drops or 100 ul of GB-Mount (GBI Cat No.E01-15: GB-Mount 15ml) to the slide and mount with coverslip. For DAB kit: Dehydrate slides in graded series of alcohol, clear in xylene. Add 2 drops or 100 ul of O-Mount (GBI Cat No. E02-15, O-Mount 15ml) to the slide and mount with coveslip. You may also use Simpo-Mount (GBI Cat. No. E03-15 or E03-100) to the slide and mount without coveslip.

Quality Control:

CONTROL SLIDES: Two control slides are essential for the interpretation of results.

1. Positive tissue control: A specimen, processed in the same way as the unknown, contains the antigen to be stained. If the positive fail to demonstrate the appropriate staining, results should be regarded as invalid.
2. Reagent Control/Negative Control: An additional slide that will be incubated with a non-immune serum instead of same concentration of primary antibody. This slide should show absence of specific staining, and provide an indication of non-specific background staining.

Trouble Shooting:

Unexpected staining results	Possible Causes	Suggested Action
Tissue section washed off	Additives in water bath did not function correctly	Remove all additives from water bath
No staining on positive slide	a. Reagents not used in correct order or chromogen mixed incorrectly	a. Refer to staining procedure
	b. Antibody incubation were skipped	b. Refer to staining procedure
	c. Improper processing of specimens	c. Refer to processing protocol
	d. Specimen drying-out during staining	d. Always buffered in PBS when procedures were interrupted

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Controls acceptable but unknown sample not stained	a. Tissue not properly prepared	Follow protocol for correct specimen preparation
	b. Unknown sample does not contain the antigen	
Weak staining on all slide	a. Diluted by retained liquid from rinsing steps	a. Blot off liquid after rinse
	b. Short incubation time	b. Increase incubation time
	c. Poor titer of primary antibody	c. Need to adjust the titer
	d. Old substrate solution	d. Change to fresh lot
Specimen staining too dark	DAB solution not properly prepared	Prepared fresh solution
Excessive background	a. Endogenous peroxidase activity not completely blocked.	a. Follow procedure for blocking peroxidase activity
	b. Paraffin not adequately removed	b. Follow procedure for deparaffin
	c. Inadequate slide rinse	c. Rinse slide completely
	d. Non-specific binding to protein	d. Use non-immune serum block
	e. Excessive amount of tissue adhesive used	e. Use less adhesive
	f. Too concentrated primary antibody	f. Re-titer primary antibody
	g. Over development of substrate	g. adjust the incubation time
No or low background on controls, but high background on sample slide	a. Sample contain nonspecific background b. Specimens not properly prepared	Follow recommended specimen preparation

Limitations

1. GBI Simplified Streptavidin HRPkit kits are provided for research or investigation use only and are not intended for therapeutic or diagnostic application. Neither Golden Bridge International, Inc. nor its sales agents shall be held responsibility for GBI Simplified Streptavidin HRPkit in a way which directly or indirectly violates local regulations or patents. Neither G
2. Golden Bridge International, Inc. nor its sales agents can be held responsible for any patent infringement which may occur as the result of improper use of this product.
3. 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.

References:

1) Elias, J.M. et al*; J Histotechnology* 15: 315-320 (1992)
2) Weaver, D.L; *J Histotechnology* 15:27-30 (1992)
3) McQuaid and Allan; *J Histochem Cytochem* 40: 569-574 (1992)
4) McMaster, M.T.*; J Immunology* 148:1699-1705 (1992)

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