GBI Labs
GOLDEN BRIDGE INTERNATIONAL

ORIGENE

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

(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 use with user-supplied primary antibody 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.

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 similar to egg white avidin. However streptavidin exhibits less non-specific binding in certain tissue specimens compared to avidin. In addition to lower background, streptavidin-biotin also demonstrates superior sensitivity to that of the Avidin-Biotin Complex (ABC) systems.

Principle:

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. Incubate tissue sections 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. 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. 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.

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

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)
Counterstain solution (Hematoxylin)
Primary antibody
10% normal goat serum in PBS
1% BSA in PBS
Chromogen/solution (AEC or DAB)
Mounting Media

Stability and Storage:

When stored at 2 – 8°C, 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 components or perform staining in direct sun light. Do not use expired reagent.

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

Precautions:

1. Do not mix reagents from different lots.
2. Do not allow the slides to dry at any time during staining.
3. Handle all specimens as potential infectious materials, wear gloves and protective clothing.
4. 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.
5. 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 fixed as soon as possible. Appropriate tissue and antigen fixation is essential to obtain reproducible and reliable results. Fixation methods may be obtained from the general literature. 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) Cell smears must be prepared to a monolayer. Cell smears are stable for one to two weeks when stored at 4°C. 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°C for 10 minutes.

2. TISSUE SECTION:

1) Section formalin fixed/processed and paraffin embedded tissue to 4μm on a standard microtome.

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.
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 to each section. Incubate for 3 -5 minutes for DAB (5 – 10min for AEC). Rinse with DI or running tap water for 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.

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com

8) **<u>Mounting Solution (not provided)</u>**: For AEC kit: apply 2 drops or 100 µl of GB-Mount (GBI Cat No.E01-18: GB-Mount 18ml) 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 µl of O-Mount (GBI Cat No. E02-18, O-Mount 18ml) to the slide and mount with coveslip. You may also use Simpo-Mount (GBI Cat. No. E03-18 or E03-100) to the slide and mount without coveslip.

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

1. Positive tissue control: A specimen expressing the antigen to be stained, processed in the same way as the unknown.
2. Reagent Control/Negative Control: A slide incubated with non-immune serum instead of primary antibody. This slide provides any 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. 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
Controls acceptable but unknown sample not stained	a. Tissue not properly prepared	Follow protocol for correct specimen preparation
	b. Unknown sample negative for the antigen	
Weak staining on all slides	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 HRP kits are provided for research or investigation use only and are not intended for therapeutic or diagnostic applications. Neither Golden Bridge International, Inc. or its sales agents shall be held responsible for use of the GBI Simplified Streptavidin HRP kit which directly or indirectly violates local regulations or patents.
2. 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)

22310 20th Ave Se #100, Bothell, WA 98021, USA Tel: 425.398.1500 Fax: 425.398.1519 http://www.gbi-inc.com