Technical Data Sheet

Streptavidin HRP

Product Information

Material Number:	**550946**
Size:	50 mL
RRID:	AB_2868972
Storage Buffer:	Aqueous buffered solution containing BSA and ProClin™ 150 as preservative.

Description

Streptavidin is a non-glycosylated protein that is purified chromatographically from the bacterium *Streptomyces avidinii*. Streptavidin homotetramers have a particularly high, non-covalent binding affinity for biotin. When conjugated with fluorochromes, streptavidin has been widely used with biotin-conjugated primary or secondary antibodies and other biotinylated specific-binding molecules to stain target molecules expressed by cells and tissues for subsequent multiparameter analysis by flow cytometry, fluorescence microscopy and imaging. When conjugated with an enzyme such as Horseradish Peroxidase (HRP) and coupled with the use of a colorimetric, luminescent, or fluorescent substrate development system, Streptavidin HRP has found widespread use along with biotinylated primary or secondary antibodies in a number of applications including Western blot, ELISA, ELISPOT, Immunocytochemistry and Immunohistochemistry.

Horseradish Peroxidase (HRP) is an enzyme that catalyzes the oxidation of various substrates in the presence of hydrogen peroxide resulting in a visible colored product. Antibodies, avidin, streptavidin and other specific binding molecules can be conjugated with HRP and used in applications such as i) ELISA that uses a plate reader to measure absorbance of the colored product, or ii) ELISPOT, Immunohistochemistry (IHC) or Western blot in which a colored product can be measured using light microscopy or image analysis.

Preparation and Storage

Streptavidin was conjugated with the enzyme under optimal conditions.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Immunohistochemistry	Routinely Tested

Recommended Assay Procedure:

Immunohistochemical Preparation and Staining Procedure for Frozen Sections

Materials needed:

Phosphate-buffered saline (PBS); 2-methylbutane (isopentane); Acetone; Liquid nitrogen: Dry ice; Hydrogen peroxide (H_2O_2); Graded alcohols; Xylene; Hematoxylin.
Peel-A-Way® Embedding Molds (Polysciences Inc., Warrington, PA).
OCT Compound for tissue freezing and embedding material (Tissue-Tek®; Sakura Finetek USA).

I. Fixation, Processing, and Sectioning of Tissue for Frozen Sections

1. Label embedding mold and partially fill the mold with tissue freezing and embedding material.
2. Place fresh tissue sample in pre-labeled embedding molds.
3. Plunge embedding mold with tissue into 2-methylbutane prechilled in a dewar of liquid nitrogen until the block ALMOST solidifies (30 seconds). NOTE: If the block is left in too long, it may crack.
4. Remove tissue block from 2-methylbutane using long forceps.
5. Place blocked tissues on dry ice. (Tissues may be stored in the embedding molds).
6. Store frozen tissue blocks in -70°C freezer until sectioning.
7. For sectioning, attach the frozen tissue block on the cryostat chuck by adhering it with a small amount of frozen tissue matrix and allow to freeze.
8. Routine sections are cut at 5 microns and picked up on a glass slide, eg, Superfrost® Plus Microscope Slides.
10. Dry overnight at room temperature (RT).
11. Fix sections in cold acetone (-20°C) for 2 min or other suitable fixative (eg, alcohol, formal alcohol, formalin, etc.).
12. Dry fixed slides completely [usually 1 hour at room temperature (RT)].
13. Store in a -70°C freezer until use.

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II. Standard Immunohistochemical Staining Procedure for Frozen Sections

Perform all incubations in a humid chamber and do not allow sections to dry out. Isotype and system controls should also be run and must be matched to the isotype of each primary antibody to be tested.

Please read entire procedure before staining slides.

1. Remove frozen slides prepared in advance from the freezer and allow to come to RT.
2. Label all slides with a pen that uses solvent resistant ink and demarcate the tissue if required.
3. Rinse slides 2-3 times in PBS to remove frozen mounting media.
4. Apply a 0.03% H_2O_2 in PBS solution (~10 min) to block endogenous peroxidase activity.
5. Rinse slides with one change PBS.
6. Wipe excess buffer from around the specimen.
7. Block with 5% normal serum diluted in PBS for 15 min.*
8. Apply primary antibody diluted in BD Pharmingen™ Antibody Diluent for IHC (Cat. No. 559148) to cover tissue sections on slide and incubate 1 hr at RT in a humid chamber.
9. Rinse slides in 3 changes of PBS, 2 min each.

Optional: Block endogenous biotin using an Endogenous Biotin Blocking Buffer, eg, Biotin/Avidin Blocking Kit (Vector Laboratories, Cat. No. SP-2001) if tissue has endogenous biotin that may cause background staining.

10. Wipe slide again and apply biotinylated secondary antibody diluted in BD Pharmingen™ Antibody Diluent for IHC and allow to incubate at RT for 30 min. This antibody must be matched to recognize the species and isotype of the primary antibody.
11. Rinse slides in 3 changes of PBS, 2 min each.
12. Wipe again and apply Strepavidin HRP using BD Pharmingen™ Streptavidin HRP (Cat. No. 550946) or a comparable commercially available Strepavidin HRP-containing solution to each slide and incubate at RT 30 min.
13. Rinse slides in 3 changes of PBS, 2 min each.
14. Prepare substrate using the BD Pharmingen™ DAB Substrate Kit (Cat. No. 550880) or alternatively AEC Substrate with the BD Pharmingen™ AEC Substrate Kit (Cat. No. 551015).
15. Drain PBS from slides, place them on a flat surface, and apply the substrate solution making sure all the section is covered by the solution. Allow slides to incubate and check the slide for color development after 5 min or until the desired color intensity is obtained. HRP activity generates a brown colored product from DAB substrate whereas HRP produces a red colored product from AEC substrate.

SAFETY NOTE: Please carefully read the associated Hazard Statements and Precautionary Statements for the substrates and components of these kits before use. DAB is a suspect carcinogen and must be handled with care.

16. Pick up each slide in the order that substrate was applied, drain excess substrate solution on paper towel and place in staining rack in a dish of water.
17. Rinse slides well in water 3 times.
18. Counterstain tissue:
    a. Dip twice in Hematoxylin.
    b. Rinse thoroughly in water.
19. Add 1-2 drops of commercially available mounting medium (eg, Aqua-Mount® Mounting Medium) on the tissue and place a glass coverslip over the tissue followed by sealing (eg, with clear nail polish) if desired.

NOTES:

* Always use serum from the species in which the secondary antibody is made: ie, if secondary antibody is Goat Anti-Mouse Ig, then block with 5% normal goat serum. Incubate for 10-30 min before application of the primary antibody. Do not rinse after this step (tap off the blocking solution if using humid chambers) and go straight to the primary antibody application.

It is important not to use Sodium Azide in any of the buffers when using Horseradish Peroxidase as azide inactivates the enzyme.

<u>Immunohistochemical Preparation and Staining Procedure for Paraffin-Embedded Sections</u>

Materials needed:

Phosphate-buffered saline (PBS); Commercially available: 10% Neutral Buffered Formalin for IHC; Hydrogen peroxide (H_2O_2); Graded alcohols; Xylene; Hematoxylin.

I. Fixation and Processing of Tissue for Paraffin Sections.

Please read entire procedure before staining slides.

A. Fixation of Tissues in 10% Neutral Buffered Formalin

1. Tissues to be fixed and processed should be cut to a size no larger than 3 mm thick. Let tissues fix in 10% Neutral Buffered Formalin at room temperature for 8 hours but not to exceed 24 hours.
2. Follow processing schedule recommended in section C.

B. Alternative -Fixation of Tissues in Zinc Fixatives:

1. Many antigenic epitopes are masked or even destroyed by 10% formalin fixation. In some cases, fixation in a milder fixative such as BD Pharmingen™ IHC Zinc Fixative (Cat. No. 550523) or BD Pharmingen™ 10X Zinc Fixative (Formalin Free) [Cat. No. 552658] is helpful to preserve the antigenic epitopes. Place fresh tissues trimmed 3 mm thick into fixative and allow tissues to fix for 24-48 hours at room temperature.
2. Follow processing schedule recommended in section C.

C. Processing Schedule: Note: The processing, embedding, and sectioning of paraffin blocks requires highly specialized equipment and expertise and is usually performed by a histology or pathology laboratory. While hand processing can be performed according to the following protocol the results may show marked variation is histology quality and antigenicity.

Station	Time	Solution
1	Delay	Treat Tissue with Fixative
2	45 min	70% Alcohol
3	45 min	80% Alcohol
4	45 min	95% Alcohol
5	45 min	100% Alcohol
6	60 min	100% Alcohol
7	60 min	100% Alcohol
8	60 min	Clearing Reagent (xylene or substitute)
9	60 min	Clearing Reagent (xylene or substitute)
10	60 min	Paraffin 1
11	60 min	Paraffin 2
12	60 min	Paraffin 3

II. Preparation of Slides with Paraffin Sections for Immunohistochemistry

A. Sectioning and Preparation of Slides.

1. Section paraffin blocks at the desired thickness (usually 4-5 µm) on a microtome and float on a water bath containing deionized or distilled water.
2. Sections are picked up on a glass slide, eg, Superfrost® Plus Microscope Slides.

B. Deparaffinization and Re-hydration of Tissue Slides:

1. Before deparaffinization, place the slides in a 55°C oven for 10 min to melt the paraffin. Deparaffinize slides in 2 changes of xylene or xylene substitute for 5 min each.
2. Transfer slides to 100% alcohol, 2 changes for 3 min each and transfer once through 95% alcohol for 3 min.
3. Block endogenous peroxidase activity by incubating sections in 3% H_2O_2 solution in methanol for 10 min.
4. Rinse in PBS 2× for 5 min each time.

Optional: If the antigen of interest is altered during the fixation process, then an antigen retrieval method can be applied. For antigen retrieval to unmask the antigenic epitope use the reagents and protocols detailed in BD Pharmingen™ Retrievagen A (pH 6.0) [Cat. No. 550524] or BD Pharmingen™ BD Retrievagen B (pH 9.5) [Cat. No. 550527].

5. Block with 5% normal serum.
6. Dilute primary antibody in Antibody Diluent for IHC (Cat. No. 559148) to cover tissue sections on slide and incubate at 4°C overnight in a humid chamber.
7. Next day, remove the slides from the refrigerator (4°C) and start with Step 9 of the ***Standard Immunohistochemical Staining Procedure***.

Note: It is important not to use Sodium Azide in any of the buffers when using Horseradish Peroxidase as azide inactivates the enzyme.

Warning: Streptavidin HRP contains 0.004% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).

Hazard statements

May cause an allergic skin reaction.

Precautionary statements

Wear protective gloves and eye protection.
Wear protective clothing.
Avoid breathing mist/vapours/spray.
If skin irritation or rash occurs: Get medical advice/attention.
IF ON SKIN: Wash with plenty of water.
Dispose of contents/container in accordance with local/regional/national/international regulations.

Suggested Companion Products

Catalog Number	Name	Size	Clone
559148	Antibody Diluent for IHC	125 mL	(none)
550880	DAB Substrate Kit	500 Tests	(none)
551015	AEC Substrate Kit	500 Tests	(none)
550523	IHC Zinc Fixative	1000 mL	(none)
552658	10X Zinc Fixative (Formalin Free)	500 mL	(none)
550524	Retrievagen A (pH 6.0)	1000 mL	(none)
550527	BD Retrievagen B (pH 9.5)	250 mL	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
4. Hazardous Ingredient: ProClin™ 150. Avoid exposure to skin and eyes and ingestion. Wash exposed skin with soap and water. Flush eyes with water.
5. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
6. ProClin is a trademark of Rohm and Haas Company.
7. For U.S. patents that may apply, see bd.com/patents.

References

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