Technical Data Sheet

AKP Streptavidin

Product Information

Material Number:	**551008**
Size:	50 ml
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Streptavidin-alkaline-phosphatase (SAv-AKP) is an enzyme used in conjunction wth a biotinylated primary or secondary antibody and an appropriate substrate for detection of an antigen by immunohistochemistry. Streptavidin has very high affinity for biotin bound to antibody and alkaline-phosphatase serves as the enzyme to produce color change in different substrates. The pre-diluted, ready-to-se SAv-AKP is convenient to use and provides optimal signal amplification and detection.

Preparation and Storage

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Immunohistochemistry	Routinely Tested

Recommended Assay Procedure:
This product is routinely tested by immunohistochemistry.

Immunohistochemistry: SAv-AKP is ready to use in its pre-diluted format. Remove the solution from 4°C and let it equilibrate to room temperature before using. The SAv-AKP should be used after the slides are incubated with biotinylated primary or secondary antibody and the unbound antibody is washed away. Apply enough SAv-AKP to cover the tissue sections and incubate 30 minutes at room temperature. Wash 3X with PBS and apply the appropriate substrate for alkaline-phosphatase (substrate BCIP/NBT). Levamisole solution may be used to block endogenous alkaline phosphatase in all tissues except gastro-intestinal tract and human placenta. Detailed protocols for immunohistochemical staining of frozen and paraffin sections are enclosed.

IMMUNOHISTOCHEMICAL STAINING FOR FROZEN SECTIONS

I. Preparation of Frozen Tissues for sectioning
Materials needed:
2-methylbutane (isopentane)
Liquid nitrogen
Dry ice
Peel-Away base molds
Frozen tissue matrix (OCT or Cryomatrix)
Long forceps
Necropsy tools
Superfrost Plus slides

1. Label base mold and partially fill the mold with frozen tissue matrix.
2. Sacrifice animal by prescribed and approved euthanasia techniques.
3. Remove desired tissues, trim and cut tissue no more than 5 mm thick. Place in pre-labeled base molds filled with frozen tissue matrix. Arrange tissue in the matrix near the bottom so tissue is easily exposed when sections are cut.
4. Place a stainless steel beaker of 2-methylbutane in liquid nitrogen and allow to cool adequately. Place base mold with tissue into the beaker of cold 2-methylbutane and quickly immerse the block. Allow the tissue matrix to solidify completely and remove block from 2-methylbutane and place on dry ice or in the -20°C cryostat. Note: If block is left in 2-methylbutane too long, the block may crack.
5. Store blocks in the -80°C freezer until ready for sectioning.

II. Sectioning of Frozen Tissues
1. Before cutting sections, allow the temperature of the block to equilibrate to the temperature of the cryostat (-20°C).

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2. Place the tissue block on the cryostat specimen disk. Adjust the positioning of the block to align the block with the knife blade. Face tissue block until the desired tissue is exposed.
3. Cut sections of the desired thickness (usually 5 µm) and place the sections on a Fisher Superfrost slide.
4. Fix slides by immersion in cold acetone (-20°C) for 2 minutes or other suitable fixative (e.g. alcohol, formal alcohol, formalin, etc.) and proceed to staining (Section III).
5. Alternately, the dry frozen section slides can be stored for a short period of time at -70°C in a sealed slide box. When ready to stain, remove slides from freezer and warm to -20°C in the cryostat or -20°C freezer, fix for 2 minutes in cold fixative (acetone or other suitable fixative) and allow to come to room temperature to continue with the staining.

III. Standard Immunohistochemical Staining Procedure For Frozen Sections

Please read entire procedure before staining 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.
Materials needed: Phosphate Buffered Saline (PBS)
Antibody diluent for IHC (Cat. No. 559148)
Streptravidin-AP
BCIP/NBTSubstrate Kit (Cat. No. 550880)

1. Label slides with a solvent resistant pen and demarcate the tissue if required.
2. Rinse slides 3 × in PBS, to remove the tissue-freezing matrix.
3. Block endogenous peroxidase activity by incubating the slides in 0.3% H_2O_2 solution in PBS for 10 minutes.
4. Rinse slides 3 × in PBS, 2 minutes each time.
5. Dilute the primary antibody in the Antibody diluent for IHC. Apply the diluted antibody to the tissue sections on the slide. Incubate for 1 hour at room temperature in a humidified chamber.
6. Rinse slides 3 × in PBS, 2 minutes each time.
7. Dilute the biotinylated secondary antibody in the Antibody diluent for IHC. Apply to the tissue sections on the slide and incubate for 30 minutes at room temperature.
8. Rinse slides 3 × in PBS, 2 minutes each time.
9. Apply the Streptravidin-AP. Pre-diluted to the tissue sections on the slide and incubate for 30 minutes at room temperature.
10. Rinse slides 3 × in PBS, 2 minutes each time.
11. Prepare BCIP/NBT substrate
12. Drain PBS from slides and apply the BCIP/NBT substrate. Allow slides to incubate for for 10-25 minutes or until the desired color intensity is obtained.
13. Wash 3 × in PBSr, 2 minutes each time.

BCIP/INT produces a reddish brown reaction product that is soluble in alcohol and therefore must be used with an aqueous counterstain and mounting media.

IMMUNOHISTOCHEMICAL STAINING FOR PARAFFIN SECTIONS

I. Fixation and Processing of Tissue for Paraffin Sections.

A. Fixation of Tissues in 10% Neutral Buffered Formalin

1. Sacrifice animal by prescribed and approved euthanasia techniques. Tissues to be fixed and processed should be cut to a size no larger than 3mm thick. Let tissues fix in 10% formalin at room temperature for 8 hours but not to exceed 24 hours. For small rodent tissue, it is recommended to fix tissues for 4-8 hours prior to processing the tissue.
2. Follow processing schedule recommended in section C.

B. Fixation of Tissues in Zinc Fixative:

1. Many antigenic epitopes are masked or even destroyed by 10% formalin fixation . In some cases fixation in a milder fixative such as Zinc fixative for IHC (New Cat. No. 550523; Previous Cat. No. 7538KZ) is helpful to preserve the antigenic epitopes. Place freshly dissected tissues trimmed 3mm thick into Zinc 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
2	45 minutes	70% Alcohol
3	45 minutes	80% Alcohol
4	45 minutes	95% Alcohol
5	45 minutes	100% Alcohol
6	60 minutes	100% Alcohol
7	60 minutes	100% Alcohol
8	60 minutes	Clearing Reagent (xylene or substitute)

9	60 minutes	Clearing Reagent (xylene or substitute)
10	60 minutes	Paraffin 1
11	60 minutes	Paraffin 2
12	60 minutes	Paraffin 3

II. Preparation of Paraffin Sections for Immunohistochemistry

A. Sectioning Protocol:
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. Transfer the sections onto a Superfrost Plus slide. Allow the slides to dry overnight and store slides at room temperature until ready for use.

B. Deparaffinization and re-hydration of tissue slide:
1. Before deparaffinization, place the slides in a 55°C oven for ten minutes to melt the paraffin. Deparaffinize slides in 2 changes of xylene or xylene substitute for 5 minutes each.
2. Transfer slides to 100% alcohol, 2 changes for 3 minutes each and transfer once through 95% alcohol for 3 minutes.
3. Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in methanol for 10 minutes.
4. Rinse in PBS 2× for 5 minutes each time.
5. If the antibody staining requires antigen retrieval to unmask the antigenic epitope refer below to section C. If antigen retrieval is not required proceed to section D.

C. Pretreatment of paraffin Sections with BD Retrievagen A (pH 6.0) (Cat. No. 550524/7539KK):
1. Make a working solution of Retrievagen A by mixing 18 ml of Retrievagen A solution 1 and 82 ml of Retrievagen A solution 2 and bring the final volume to 1 liter in distilled water.
2. Place slides in a plastic coplin jar filled with the working Retrievagen solution and heat in a microwave oven to 193°F; (89 °C) (microwave oven * or other heating sources such as pressure cooker, water bath can be used).
3. Mix the working Retrievagen A solution in the coplin jar with a disposable pipet and incubate the slides at 193°F; for 10 minutes.
4. Remove the coplin jar with the slides, cover the jar tightly, and allow the solution to slowly cool to room temperature for 20 minutes.
Note: It is important to let the temperature to slowly ramp down to allow the protein molecules to fold properly.
5. Rinse slides in PBS 3×, 5 minutes each time.
Note: *Heating by use of microwave oven may require a license under US patent No. 5244787.

D. Immunohistochemical staining of paraffin embedded tissues
Refer to "Standard Immunohistochemical Staining Procedure" (Section III of Immunohistochemical staining of frozen sections).
Begin at step 5 and proceed through coverslipping.

Suggested Companion Products

Catalog Number	Name	Size	Clone
559148	Antibody Diluent for IHC	125 ml	(none)
550523	IHC Zinc Fixative	1000 ml	(none)
550524	Retrievagen A (pH 6.0)	1000 ml	(none)
550880	DAB Substrate Kit	500 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.