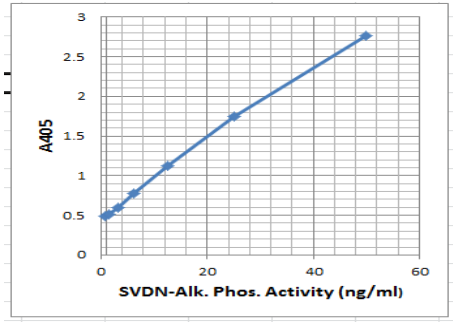
ASSAY PROTOCOL

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

- Add **50ul of diluted SVDN-AP stds** (A-F) Conjugate in duplicate to appropriate wells. 50 ul conjugate diluent (blank) and samples to appropriate wells.
- Gently mix for 5-10 seconds, cover the plate and Incubate for 30 minutes at room temp (25-28oC) or use an incubator set at 25-28oC.
- **Wash wells 5 times** using 300 ul/well for each wash; tap over the plate on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.
- Add **50 ul AP Substrate** to each well. Gently mix for 5-10 seconds, cover the plate and Incubate for **15 minutes at RT** (25-28oC). The liquid in the wells will begin to turn yellow.
- Add **50ul of Stop Solution** to each well. Tap gently to mix. The enzyme reaction will stop and color remains yellow.
- Read the plate at 405nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 405nm using a single wavelength within 60 minutes after Stop Solution addition.

Typical Calibrator Curve

Well positions	Stds/Sample	A405 (average)
A1/A2	50 ng/ml (std F)	2.768
B1/B2	25 ng/ml (std E)	1.757
C1/C2	12.5 ng/ml (std D)	1.125
D1/D2	6.25 ng/ml (std C)	0.779
E1/E2	3.125 ng/ml (std B)	0.592
F1/F2	1.56 ng/ml (std A)	0.518
G1/G2	samples	



1-Va-ELISA-Graphs

Sensitivity
Streptavidin-AP was detected at concentrations significantly above background in an ELISA format using pNPP as substrate (at 6.25pg/ml).

Instruction Manual No. M-80305-SAP

Streptavidin Alkaline Phosphatase Activity Elisa kit

ELISA Kit Cat. No. 80305-SAP, 48 tests

For detection of Streptavidin and Alkaline Phosphatase Activity

GENEMED SYNTHESIS, INC.
6203 Wood Lake Center Dr., Bldg. 2, San Antonio, TX 78244, USA
Toll free (800) 344-5337; **Phone**: (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, Website: www.genemedsyn.com

Streptavidin Alkaline Phosphatase Activity Elisa kit, 48 tests

ELISA Kit Components	Part No.	Size
Biotin coated Strip Plate, 8-well strips (6)	80305-BT	48 wells
Streptavidin-AP Reference standard (lot sp. Conc on the vial), 55 ul (100X)	80305-SAC	1 vial
AP Conjugate Diluent buffer, 3ml	80305-CD	1 bottle
Wash Buffer Concentrate (50X), 5ml	WB-50	1 bottle
AP Substrate, 5ml	80305-AP	1 bottle
Stop Solution, 5ml	80305-ST	1 bottle
Product Manual	80305-SAP	1 ea
Store whole kit at 2-4oC. Expiration is 1 year.		

INTENDED USE

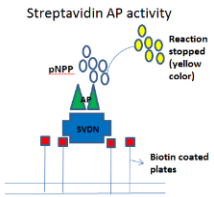
The Streptavidin-Alkaline Phosphatase (SVDN-AP) Activity Elisa kit is a direct test suitable for detecting the functioning or activity of both streptavidin and alkaline phosphatase in the conjugates. The ELISA tests is based upon streptavidin binding to biotin-coated plates followed by the detection of enzyme activity using highly sensitive substrate. The test contains all reagents and performed at room temperature in 45 minutes. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

GENERAL INFORMATION

Streptavidin is a 53 Kda tetrameric protein purified from the bacterium Streptomyces avidinii. It finds wide uses in immunoassay and molecular biology due to its extraordinarily strong affinity for the vitamin biotin; the dissociation constant (Kd) of the biotin-streptavidin complex is on the order of ~10-15 mol/L, ranking among one of the strongest known non-covalent interactions. Streptavidin's affinity for biotin is exploited in wide ranging biochemical assays, including western blot, ELISA, ELISPOT and pull-down assays. Streptavidin immobilized onto solid supports (ELISA plates, agarose, nitrocellulose etc) is also used as purification media to capture biotin-labelled protein or nucleic acid molecules. For example, cell surface proteins can be specifically labelled with membrane impermeable biotin reagent, then specifically captured using an avidin-based support. **Alkaline phosphatases (APs)** are highly ubiquitous enzymes, present in all species from bacteria to man. In vitro, the enzymes behave as phosphotransferases at neutral pH. The use of phosphate acceptor molecules (diethanolamine, tris, 2-amino-2-methyl-1- propanol) in the buffered substrate solutions increases the reaction rates and, thus, the sensitivity of assays based on AP determinations. AP is commonly used as a label in immunoassays such as ELISA, and in blotting and histochemistry. Once conjugated to antibodies, antigens, or streptavidin, its low backgrounds and linear reaction rate enables increased sensitivity over extended incubation times. It can be used with a variety of substrates producing precipitated or soluble chromogens, or with chemiluminescent substrates for enhanced sensitivity.

Streptavidin can be coupled to commonly used enzymes (Horseradish peroxidase/HRP or Alkaline phosphatase/AP) to produce general purpose detection system to detect biotinylated proteins or antibodies. It is necessary to determine the functionally of streptavidin-AP conjugate for determining an appropriate concentration in various immunoassays (ELISA, Western blot, and Immunohistochemistry).

PRINCIPLE OF THE TEST



The SVDN-AP test is based on the binding of SVDN to biotin immobilized on the ELISA plate. SVDN-AP conjugate is reacted with the plate for 30 min at room temp and unbound conjugate is removed by washing. Highly sensitive chromogenic AP substrate (pNPP) is added and color (yellow) is developed by the enzymatic reaction of AP on the substrate. Stopping Solution is added to terminate the reaction, and the (405nm) is then measured using an ELISA microwell reader. Amount of color is directly proportional to the amount of AP present in the sample. A reference SVDN-AP conjugate is provided to compare the activity of unknown samples.

Reagent Preparation

Wash Buffer Concentrate (50x) Dilute the entire volume 5ml + 250ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at ambient temperature until kit is used entirely.

Streptavidin-AP Reference Conjugate (100x) Part No. 80305-SAC, 0.05ml
Phosphatase coupled to Streptavidin. Dilute fresh as needed; 5ul of concentrate to 495ul of conjugate Diluent is sufficient for the first well. Use within the working day and discard. Return 100X to 2-8oC storage.

The SVDN-AP conjugate is supplied at 100X Stock with an estimated AP activity of (166 ng/ml). Prepare additional standards (A-E) in microtube vials as follows. Use the AP-conjugate diluent to prepare additional standards (2-fold serial dilutions). Prepare as needed and do not working standards beyond the assay date. You will use 50 ul x 2 (duplicate) to run the test.

Stds.		Conj. Diluent	Volume	Final Conc of AP (ng/ml)
F	5 ul of SVDN-AP (100X)	495 ul	500 ul	50
E	125 ul of std. F	125 ul	250 ul	25
D	125 ul of std. E	125 ul	250 ul	12.5
C	125ul of std. D	125 ul	250 ul	6.25
B	125 ul of std C	125 ul	250 ul	3.125
A	125 ul of std. B	125 ul	250 ul	1.56
Blanks	-	125 ul	125 ul	

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting conjugates.
- Graduated cylinder to dilute Wash Concen. and Sample Diluent concentrate; 200ml to 1L.
- Distilled or deionized water, Microwell plate reader at 405 nm wavelength.

Note: Stop Solution contains diluted NaOH. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

LIMITATIONS OF THE ASSAY

Quantitation of SVDN-AP in a Sample conjugate

- The ELISA requires active streptavidin and AP. Loss of activity in either will results in decreased sensitivity or no detection. The SVDN- AP conjugate must not be diluted in a buffer that affect the activity of the conjugate.
- The kit will not detect free SVDN or Alka. Phos.

ASSAY DESIGN AND SET-UP

- Bring all reagents to room temperature (25-28^{o} C) equilibration (at least 30 minutes).
- Prepare **SVDN-AP conjugate** standards A-E in AP-conjugate Diluent (see page 2).
- Determine the number of wells for the assay run. Duplicates are recommended, including 7 standards wells and 1 wells for each sample diluent (blanks) and samples to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.