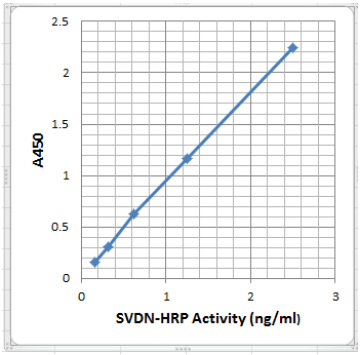
ASSAY PROTOCOL

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-HRP 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 TMB 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 blue.
- Add **50ul of Stop Solution** to each well. Tap gently to mix. The enzyme reaction will stop and color turns yellow.
- Read the plate at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 60 minutes after Stop Solution addition.

Typical Calibrator Curve

Well positions	Stds/Sample	A450/630 (average)
A1/A2	2.5 ng/ml (std E)	2.244
B1/B2	1.25 ng/ml (std D)	1.165
C1/C2	0.625 ng/ml (std C)	0.628
D1/D2	0.312 ng/ml (std B)	0.312
E1/E2	0.156 ng/ml (std A)	0.160
F1/F2	samples	



1-Va-ELISA-Graphs

Sensitivity
Streptavidin-HRP was detected at concentrations significantly above background in an ELISA format using TMB as substrate (at 0.156 ng/ml).

Instruction Manual No. M-80306-SRP

Streptavidin Alkaline Horse radish peroxidase (HRP) Elisa kit

ELISA Kit Cat. No. 80306-SRP, 48 tests

For detection of Streptavidin and Horse radish peroxidase 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 Horseradish peroxidase Activity Elisa kit, 48 tests

ELISA Kit Components	Part No.	Size
Biotin coated Strip Plate, 8-well strips (6)	80306-BT	48 wells
Streptavidin-HRP Reference standard (lot sp. Conc on the vial), 55 ul (100X)	80306-SRC	1 vial
Conjugate Diluent buffer, 3ml	80306-CD	1 bottle
Wash Buffer Concentrate (50X), 5ml	WB-50	1 bottle
TMB Substrate, 5ml	80306-TMB	1 bottle
Stop Solution, 5ml	80306-ST	1 bottle
Product Manual	80306-SRP	1 ea
Store whole kit at 2-4oC. Expiration is 1 year.		

INTENDED USE

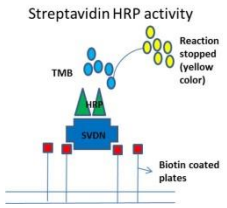
The Streptavidin-peroxidase (SVDN-HRP) Activity Elisa kit is a direct test suitable for detecting the functioning or activity of both streptavidin and horseradish peroxidase in the conjugates. The ELISA tests is based upon streptavidin binding to biotin-coated plates followed by the detection of enzyme 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**. Horseradish peroxidase (HRP)** is isolated from horseradish roots (Amoracia rusticana) and belongs to the ferroprotoporphyrin group of peroxidases. HRP is a single chain polypeptide containing four disulfide bridges. It is a glycoprotein containing 18% carbohydrate. Its molecular weight (approx. 44 kDa) includes the polypeptide chain (33,890 Daltons), hemin plus Ca2+ (approx. 700 Daltons), and carbo-hydrate (9400 Daltons). The isoelectric point for horseradish Peroxidase isozymes ranges from 3.0 - 9.0. HRP readily combines with hydrogen peroxide (H2O2) and the resultant [HRP-H2O2] complex can oxidize a wide variety of chromogenic hydrogen donors. It can also utilize chemiluminescent substrates such as luminol and isoluminol and fluorogenic substrates such as tyramine, homovanillic acid, 4-hydroxyphenyl acetic acid. The following compounds are inhibitors of horseradish peroxidase: sodium azide, cyanide, L-cystine, dichromate, ethylenethiourea, hydroxylamine, sulfide, vanadate, paminobenzoic acid. The enzyme is most stable in the pH range of 5.0 to 9.0.5

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-HRP conjugate for determining an appropriate concentration in various immunoassays (ELISA, Western blot, and Immunohistochemistry).

PRINCIPLE OF THE TEST



The SVDN-HRP test is based on the binding of SVDN to biotin immobilized on the ELISA plate. SVDN-HRP conjugate is reacted with the plate for 30 min at room temp and unbound conjugate is removed by washing. Highly sensitive chromogenic substrate (TMB) is added and color (blue) is developed by the enzymatic reaction of HRP on the substrate. Stopping Solution is added to terminate the reaction, and the (450nm) is then measured using an ELISA microwell reader. Amount of color is directly proportional to the amount of peroxidase enzyme present in the sample. A reference SVDN-HRP 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-HRP Reference Conjugate (100x) Part No. 80306-SAC, 0.05ml
Peroxidase 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-HRP conjugate is supplied at 100X Stock with an estimated HRP activity of (166 ng/ml). Prepare additional standards (A-E) in microtube vials as follows. Use the 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 HRP (ng/ml)
F	5 ul of SVDN-HRP (100X)	495 ul	500 ul	5
E	125 ul of std. F	125 ul	250 ul	2.5
D	125 ul of std. E	125 ul	250 ul	1.25
C	125ul of std. D	125 ul	250 ul	0.625
B	125 ul of std C	125 ul	250 ul	0.312
A	125 ul of std. B	125 ul	250 ul	0.156
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 450 nm wavelength.

Note: Stop Solution contains diluted sulfuric acid. 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-HRP in a Sample conjugate

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

ASSAY DESIGN AND SET-UP

- Bring all reagents to room temperature (25-28^{o} C) equilibration (at least 30 minutes).
- Prepare **SVDN-HRP conjugate** standards A-E in 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.