INTENDED USE

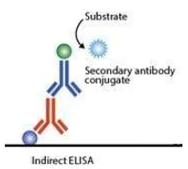
Horse Anti-Saw Scaled Viper antibody tests is an indirect ELISA suitable for detecting antivenin IgG in horse serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. This kit is particularly designed to assess the immunological potency or antibody concentration of the anti-venom (monovalent or polyvalent) produced in horse. For *in vitro research use only (RUO), not for therapeutic or diagnostic use*.

GENERAL INFORMATION

Snake venom is highly modified saliva containing zootoxins used by snakes to immobilize and digest prey or to serve as a defense mechanism against a potential predator or other threat. Venoms contain more than 20 different compounds, 100s proteins and polypeptides. **Envenomation** is the process by which venom is injected into animals and humans. Although the majority of snake species are non-venomous and typically kill their prey with constriction rather than venom, venomous snakes can be found on every continent except Antarctica. The morbidity and mortality associated with snake bites is a serious public health problem in many regions of the world.

Antivenom (or antivenin or antivenene) is a biological product used in the treatment of venomous bites or stings. Antivenom is created by milking venom from the desired snake, spider or insect. The venom is then diluted and injected into a horse, sheep or goat (antivenom host). The subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecule which can then be harvested from the animal's blood and used to treat envenomation. Antivenoms can be classified into **monovalent** (when they are effective against a given species' venom) or **polyvalent** (when they are effective against a range of species, or several different species at the same time). Antivenom (whole antiserum from horse (equine), sheep (ovine), goat (caprine) or chicken is usually purified to remove most serum proteins leaving mostly immunoglobulin (Ig's). Whole crude antibodies may also be subjected to antibody fragmentation to prepare only the **Fab2 fragments** of the antibodies to minimize exposure to the foreign proteins to minimize subsequent hypersensitivity reaction (anaphylaxis) or a delayed hypersensitivity (serum sickness). In the U.S. the only approved antivenom for pit viper (rattlesnake, copperhead and water moccasin) snakebite is based on a purified product made in sheep known as **CroFab** (Crotalidae Polyvalent Immune Fab (Ovine/Sheep)) is the only widely available antivenom indicated for the management of patients with minimal to moderate North American Crotalid envenomation (rattlesnake, water moccasin/cottonmouth and copperhead

PRINCIPLE OF THE TEST



The Horse Anti-snake antibody ELISA kit is based on the binding of antibody in samples to purified antigens (venom) coated on the plate, and antibody is detected by species specific antibody conjugated to HRP. After a washing step, substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of antibody present in the sample. Stop Solution is added to terminate the reaction (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The presence or concentration of antibody in samples is determined relative to supplied controls or calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (50x) Cat. #WB-50, 10 ml	Dilute the entire volume 10ml + 490 ml with distilled or deionized water into a clean stock bottle. Label as ***1X* Wash Solution** and store at 4°C for long term and ambient temp. for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** (WSD) and store at 2-8°C until the kit lot expires or is used up.
Anti-Horse IgG-Fab2 HRP Conjugate Concentrate (100x) Part: 570132, 0.11ml	in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
SSV Venom antigen coated Plate	570131	8-well strips (12)	Coated with Saw Scaled viper venom antigens, and post-coated with stabilizers.
Horse Anti-Saw Scaled Viper antibody calibrators			
3 U/ml **10 U/ml** **30 U/ml** **100 U/ml**	570133A 570133B 570133C 570133D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Supplied in a buffer with protein, detergents and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

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 samples and Anti-Horse IgG HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability and Sample dilution

Initial dilution of serum into **Working Sample Diluent** (1X WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Hyperimmune anti-venom are generally high tittered and they may require a dilution between 1:1000-1:100.000 or more. We suggest the following scheme to make antisera dilutions.

1. Make an initial 1:100 dilution in 1X WSD (5 ul sample in 495 ul diluent). Use this stock to make all test dilution (1:1000-1:100,000).
2. Make test dilutions from 1:100 stock (5 ul of 1:100 in 495 ul of 1X WSD; final dilution 1:10,000)
3. Make test dilutions from 1:10,000 stock (25 ul of 1:10,000 in 225 ul of 1X WSD; final dilution 1:100,000)

Test a few dilutions of given samples to see what dilution is required to bring them into testing range of the ELISA.

Assay Design

Review Calculation of Results (p5-7) and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding and other matrix effects; for example, net signal for non-immune samples should be lower than the **3 U/ml or user specified cut-off values.**
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.

Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 4 Control wells and 2 wells for each sample and internal control 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.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

Assay Procedure

- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

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

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of 1X WSD (blank), calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry 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.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Horse IgG-HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

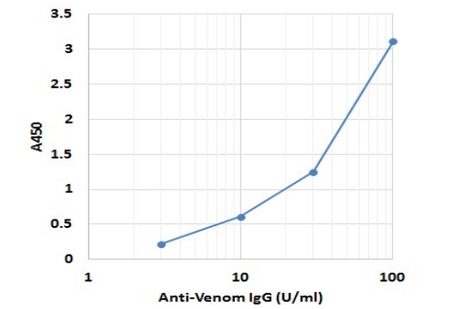
PRECAUTIONS AND SAFETY INSTRUCTIONS

Controls, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website:http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

INTERPRETATION OF RESULTS

Wells	Stds/samples	U/mL	Mean A450	Net A450
A1, A2	**Blank**	0.0	0.04	-
B1, B2	Calibrator A	3	0.22	0.18
C1, C2	Calibrator B	10	0.61	0.57
D1, D2	Calibrator C	30	1.25	1.21
D1, D2	Calibrator D	100	3.11	3.07

Example:



Horse Anti-Saw Scaled Viper Venom IgG ELISA

3-soum/570-130-SHG-Horse-Anti-Saw Scaled Viper-venom-IgG-ELISA-Graph

Calculations

1. Subtract blank values from all values (standards, controls and samples) to calculate the net A450. Draw a semi-log graph as depicted above. We recommend using point-to-point curve or 4-point curve.
2. Determine the concentration of the unknown from the graphs and multiply the conc with the sample dilution.
3. Results are expressed as Units/ml (U/ml). Standards are prepared from in-house sera that are assigned arbitrary units. In general, 100 Units correspond to approximately 100 ng antibody. However, antibody reactivity to different antigens (venom) may alter this concentration.
4. All samples that are outside the range of the standards should be tested at a dilution that will be within the range of the standards.

Note: A commercially available horse anti-venom Fab2 polyvalent preparation was tested (VINS bio #01AS13003; ADI 100 units =1:5000 dilution of Saw scaled Viper venom antibody). Concentration of antibodies may differ in various lots in any ELISA. We recommend that users include an internal control with defined concentration of anti-venom activity.

PRODUCT SPECIFICATIONS

Echis carinatus, commonly called the saw-scaled viper (Indian saw-scaled viper, little Indian viper), is a venomous viper species found in parts of the Middle East and Central Asia, and especially the Indian subcontinent. It is the smallest member of the Big Four snakes that are responsible for causing the most snakebite cases and deaths, due to various factors including their frequent occurrence in highly populated regions, and their inconspicuous nature. Five subspecies are currently recognized. The venom from this species is used in the manufacture of several drugs. One is called echistatin, which is an anticoagulant. Even though many other snake venoms contain similar toxins, echistatin is not only especially potent, but also simplistic in structure, which makes it easier to replicate. Indeed, it is obtained not only through the purification of whole venom,[16] but also as a product of chemical synthesis. Another drug made from E. carinatus venom is called ecarin and is the primary reagent in the ecarin clotting time (ECT) test, which is used to monitor anticoagulation during treatment with hirudin. Yet another drug produced from E. carinatus venom is Aggrastat (Tirofiban).

Antigen, Antibody Specificity & Sensitivity

Saw scaled viper antigens (venom) is used as antigen for the detection of antibodies. Therefore, this kit detects antibodies to all venom proteins. The antigen coating level, HRP conjugate concentration, and sample Diluent are optimized to differentiate anti-venom antibody from background (non-antibody) signal with horse serum samples at an appropriate dilution. The kit uses antibody calibrator with arbitrary units/ml (100 U/ml represents ~ 100 ng/ml horse IgG) The lowest limit of detection is about 0.3 ng of Horse IgG.

This kit is designed to detect the anti-venom IgG produced against the saw scaled venom (monovalent) or polyvalent in horse. The antibody conjugate used in the kit mainly detect the **IgG-Fab2** but some cross detection of IgM or other isotype may be observed. This kit can be used to detect the horse anti-saw scaled antivenom IgG in unpurified antisera, partially purified whole IgG or purified Fab2.

References: https://en.wikipedia.org/wiki/Echis_carinatus; McDiarmid RW (1999) Snake Species of the World: A Taxonomic and Geographic Reference, Volume 1. Washington, District of Columbia: Herpetologists' League. 511 pp; Echis carinatus". Integrated Taxonomic Information System. Retrieved 1 August 2006.

Related Items

Catalog#	Description
570-100-CHG	Horse Anti-Cobra (Naja Naja) Antibody ELISA Kit, 96 tests, Quantitative, 96 tests
570-110-KHG	Horse Anti-Indian Krait (Bungarus Caeruleus) Antibody ELISA Kit, 96 tests, Quantitative
570-120-RHG	Horse Anti-Russell's Viper (Vipera Russelli) Antibody ELISA Kit, 96 tests, Quantitative
570-130-SHG	Horse Anti-Saw Scaled Viper (Echis Carinatus) Antibody ELISA Kit, 96 tests, Quantitative
570-140-XHG	Horse Anti-Common (Cobra, Crait, Russels and Saw scaled vipers) Antibody ELISA Kit, 96 tests, Quantitative
570-200-DSG	Sheep Anti-Diamond-back Rattlesnake (Crotalus atrox) Antibody ELISA Kit, 96 tests, Quantitative
570-210-CSG	Sheep Anti-Pit Viper Copperhead (Agkistrodon contortrix) Antibody ELISA Kit, 96 tests, Quantitative
570-220-CSG	Sheep Anti-Water Moccasin/cottonmouth pit viper (Agkistrodon piscivorus) Antibody ELISA Kit, 96 tests, Quantitative
7610-Fab	Sheep/Ovine Fab ELISA Kit, 96 tests, Quantitative
7615-Fc	Sheep IgG-Fc ELISA Kit, 96 tests, Quantitative
7710-Fab	Horse Fab2 ELISA Kit, 96 tests, Quantitative
APVS11-S	Rabbit Anti-Black Mocassin (Agistron piscovirus) venom
APVS12-S	Chicken Anti-Black Mocassin (Agistron piscovirus) venom
APVS14-S	Sheep Anti-Black Mocassin (Agistron piscovirus) venom
CADM11-S	Rabbit Anti-Eastern Diamondback venom antiserum
CADM12-S	Chicken Anti-Eastern Diamondback venom antiserum
CADM14-S	Sheep Anti-Eastern Diamondback venom antiserum
CATX11-S	Rabbit Anti-Western Diamondback Rattlesnake venom antiserum
CATX12-S	Chicken Anti-Western Diamondback Rattlesnake venom antiserum
CATX14-S	Sheep Anti-Western Diamondback Rattlesnake venom antiserum
CATX15-S	Rabbit Anti-Common N. American (Diamondback, copperhead and Mocacassin snakes) venom antiserum
CATX16-S	Chicken Anti-Common N. American (Diamondback, copperhead and Mocacassin snakes) venom antiserum
CATX17-S	Sheep Anti-Common N. American (Diamondback, copperhead and Mocacassin snakes) venom antiserum
CKT11-S	Rabbit Anti-Indian krait (Bungarus caeruleus) venom antiserum
CKT12-S	Chicken Anti-Indian krait (Bungarus caeruleus) venom antiserum
CKT13-S	Horse Anti-Indian krait (Bungarus caeruleus) venom antiserum
ICO11-S	Rabbit Anti-Indian Cobra (Naja naja) venom antiserum
ICO12-S	Chicken Anti-Indian Cobra (Naja naja) antiserum
ICO13-S	Horse Anti-Indian Cobra (Naja naja) venom antiserum
RVR11-S	Rabbit Anti-Russell's Viper (Vipera russelli) venom antiserum
RVR12-S	Chicken Anti-Russell's Viper (Vipera russelli) venom antiserum
RVR13-S	Horse Anti-Russell's Viper (Vipera russelli) venom antiserum
SSV11-S	Rabbit Anti-Saw-scaled Viper venom antiserum
SSV12-S	Chicken Anti-Saw-scaled Viper venom antiserum
SSV13-S	Horse Anti-Saw-scaled viper venom antiserum
VNM11-S	Rabbit Anti-Common Asian (Cobra, Crait, Russell's and Saw-scaled vipers) venom antiserum
VNM12-S	Chicken Anti-Common Asian (Cobra, Crait, Russell's and Saw-scaled vipers) venom antiserum
VNM13-S	Horse Anti-Common Asian (Cobra, Crait, Russell's and Saw-scaled vipers) venom antiserum

Instruction Manual No. 570-130-SHG

Horse Anti-Saw Scaled Viper (Echis Carinatus) IgG ELISA Kit, 96 tests

Cat. #. 570-130-SHG, 96 Tests

For the quantitation saw scaled viper antibody horse serum, plasma or other biological fluids

For in vitro research use only (RUO), not for therapeutic or diagnostic use.

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive•San Antonio•Texas78244•USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
service@4adi.com
www.4adi.com