INTENDED USE

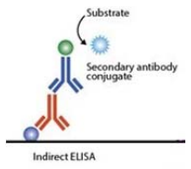
Horse Anti-Russell's Viper Venom Antibody test is an indirect ELISA suitable for detecting antivenom IgG-$(Fab')_2$ in horse serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. This kit is particularly designed to assess the immunological potency or antibody 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 **$(Fab')_2$ 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 Venom Antibody** ELISA kit is based on the binding of antibody in samples to purified venom coated on the plate, and antibody is detected by Anti-Horse IgG-$(Fab')_2$ 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 calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°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 (100x) Cat. #WB100, 10 ml	Dilute the entire volume 10ml + 1L distilled or deionized water into a clean stock bottle. Label as ***1X* Wash Solution** and store refrigerated for long term and ambient temperature 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-$(Fab')_2$-HRP Conjugate Concentrate (100x) Part 7714, 0.15ml	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
Russells Viper Venom coated Plate	570121	8-well strips (12)	Coated with Indian Cobra venom, and post-coated with stabilizers.
Anti-RVR Venom Calibrators			
5 U/ml **15 U/ml** **45 U/ml** **100 U/ml**	570122B 570122C 570122D 570122E	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 titered and 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 **5 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.
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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]

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

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

3. Substrate Incubation [100ul – 15 min]

- o Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- o 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]

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

5. Absorbance Reading

- o 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.
- o 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.

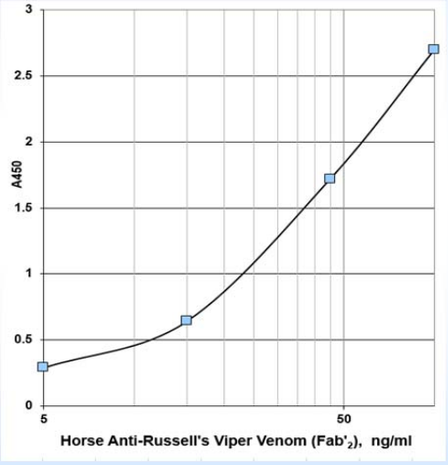
PRECAUTIONS AND SAFETY INSTRUCTIONS

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

INTERPRETATION OF RESULTS

1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, horse IgG concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the Calibrators (y-axis) against the concentration (U/ml) of horse IgG (x-axis). Draw the best fit curve through these points to construct the Calibrator curve. A point-to-point construction is most common and reliable.
4. The horse IgG concentrations in unknown samples and controls can be determined by interpolation from the Calibrator curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 100 U/ml Calibrator should be further diluted and re-assayed.

Wells	Calibrators & Samples		A450 nm
A1, A2	Diluent Blank		0.04
B1, B2	**5** U/ml	**Calibrator**	0.34
C1, C2	**15** U/ml	**Calibrator**	0.90
D1, D2	**45** U/ml	**Calibrator**	1.88
E1, E2	**100** U/ml	**Calibrator**	2.66



Note: A commercially available horse anti-venom $(Fab')_2$ polyvalent preparation was tested (VINS bio #01AS13003; ADI 100 units =1:10,000 dilution of Russell's 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

Daboia russelli is commonly known as Russell's viper and chain viper, among other names. Daboia is a monotypic genus of venomous Old World vipers. The single species, D. russelii, is found in Asia throughout the Indian subcontinent, much of Southeast Asia, southern China and Taiwan, and Pakistan. Apart from being a member of the big four snakes in India, Daboia is also one of the genera responsible for causing the most snakebite incidents and deaths among all venomous snakes on account of many factors, such as their wide distribution, generally aggressive demeanor, and frequent occurrence in highly populated areas.

The quantity of venom produced by individual specimens is considerable. Reported venom yields for adult specimens range from 130–250 mg to 150–250 mg to 21–268 mg. The LD50 in mice, which is used as a possible indicator of snake venom toxicity, is: 0.133 mg/kg intravenous, 0.40 mg/kg intraperitoneal, about 0.75 mg/kg subcutaneous. For most humans, a lethal dose is about 40–70 mg. In general, the toxicity depends on a combination of five different venom fractions, each of which is less toxic when tested separately. Venom toxicity and bite symptoms in humans vary within different populations and over time. Envenomation symptoms begin with pain at the site of the bite, immediately followed by swelling of the affected extremity. Bleeding is a common symptom, especially from the gums and in the urine, and sputum may show signs of blood within 20 minutes after the bite. In India, the Haffkine Institute prepares a polyvalent antivenin that is used to treat bites from this species.

Venom is very effective at inducing thrombosis, it has been incorporated into an in vitro diagnostic test for blood clotting that is widely used in hospital laboratories. This test is often referred to as dilute Russell's viper venom time (dRVVT). The coagulant in the venom directly activates factor X, which turns prothrombin into thrombin in the presence of factor V and phospholipid. The venom is diluted to give a clotting time of 23 to 27 seconds and the phospholipid is reduced to make the test extremely sensitive to phospholipid. The dRVVT test is more sensitive than the aPTT test for the detection of lupus anticoagulant (an autoimmune disorder), because it is not influenced by deficiencies in clotting factors VIII, IX or XI.

Antigen, Antibody Specificity & Sensitivity

Russell's Viper antigens (venom) is used as antigen for the detection of antibodies. Therefore, this kit detects antibodies to the venom protein. 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 $(Fab')_2$.

References: https://en.wikipedia.org/wiki/Russell%27s_viper; 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. August 2006.

NOTES

Instruction Manual No. 570-120-RHG

Horse Anti-Russell's Viper (*Daboia russelli*) Venom Antibody ELISA Kit

Cat. #. 570-120-RHG, 96 Tests

For the quantitation of Russell's Viper venom antibody in horse serum, plasma or other biological fluids

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

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