KRISHGEN BioSystems

KRIBIOLISA™ Desmopressin (Minirin™, Nocdurna™, DDAVP™) ELISA

REF : KBI5010

Ver 2.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Desmopressin in human serum and plasma

RUO	**For Research Use**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF KBI5010 Σ 96 tests

KRISHGEN BioSystems | For US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005
For Asia / India: tel: +91(22)-49198700
Email: sales@krishgen.com

Introduction:

Desmopressin (Minirin™, Nocdurna™, DDAVP™) is a medication used to treat diabetes insipidus, bedwetting, hemophilia A, von Willebrand disease, and high blood urea levels. Desmopressin (dDAVP), a synthetic analogue of 8-arginine vasopressin (ADH), is an antidiuretic peptide drug modified by deamination of 1- cysteine and substitution of 8-L-arginine by 8-D-arginine. ADH is an endogenous pituitary hormone that has a crucial role in the control of the water content in the body. Upon release from the stimulation of increased plasma osmolarity or decreased circulating blood volume, ADH mainly acts on the cells of the distal part of the nephron and the collecting tubules in the kidney. The hormone interacts with V1, V2 or V3 receptors with differing signal cascade systems.

Intended Use:

The KRIBIOLISA™ Desmopressin (Minirin™, Nocdurna™, DDAVP™) ELISA is used as an analytical tool for the quantitative determination of desmopressin in human serum and plasma.

Principle:

The KRIBIOLISA™ Desmopressin (Minirin™, Nocdurna™, DDAVP™) is a competitive inhibition immunoassay for the determination of Desmopressin. Anti-DDAVP monoclonal antibody are pre-coated onto the microwells. A constant concentration of Biotin Concentrate and varying concentrations of Standard or Sample compete for binding / inhibiting specifically to the coated antibodies. Then, Streptavidin:HRP conjugate is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops in inverse proportion to the amount of Desmopressin in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Anti-DDAVP Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-DDAVP monoclonal antibody.	1 x 96 wells
Desmopressin Standard	Desmopressin in a buffered protein base (concentrated, 33.3 ug/ml, 0.5 ml each)	2 vials
Biotinylated DDAVP	Biotinylated DDAVP with protein stabilizer and preservative sodium azide <0.01%	1 vial
Streptavidin:HRP Conjugate	Streptavidin:HRP with protein stabilizer and preservative sodium azide <0.01%	1 vial
(1X) Sample Diluent	Buffered protein base with preservative thiomersol < 0.01%	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with 1:1000 dilution human serum and preservative sodium azide < 0.01%	10 ml
Reagent Diluent	Buffered protein base with preservative sodium azide < 0.01%	300 ul
Assay Diluent A	Buffered protein base with preservative thiomersol < 0.01%	12 ml
Assay Diluent B	Buffered protein base with preservative thiomersol < 0.01%	12 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis

6. Timer

7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation – Serum and Plasma samples have to be diluted 1:1000 (v/v), e.g. 1 ul sample + 999 ul Sample Diluent prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of **(20X) Wash Buffer** in 475 ml of DI water.
4. **Biotinylated DDAVP** - Reconstitute **Biotinylated DDAVP** with 150 ul of **Reagent Diluent**. Keep for 10 minutes at room temperature, with intermittent gentle shake (not to foam). Dilute to the working concentration with **Assay Diluent A** (1:100).
5. **Streptavidin:HRP Conjugate** - Briefly spin or centrifuge the stock **Streptavidin:HRP** before use. Dilute to the working concentration with **Assay Diluent B** (1:100)
6. **Standards Preparation**: Keep the **original standard** be at RT for 15 minutes. Dilute 300.3 ul of **original Standard (33.3 ug/ml)** with 699.7 ul of Standard Diluent to generate a **10,000 ng/ml Standard Solution**. This is the top standard. Prepare further **Standards** by diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
33.3 ug/ml	Original Standard	Orignal standard provided in kit
10,000 ng/ml	Standard No.6	300.3 ul Original Standard (33.3ug/ml) + 699.7 ul Standard Diluent
7000 ng/ml	Standard No.5	700 ul Standard No.6 + 300 ul Standard Diluent
5000 ng/ml	Standard No.4	714.3 ul Standard No.5 + 285.7 ul Standard Diluent
1000 ng/ml	Standard No.3	200 ul Standard No.4 + 800 ul Standard Diluent
500 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
250 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No.0	Only Standard Diluent

Discard balance standard after use. Do not store them for further experiments

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Desmopressin. High Dose Hook Effect is due to excess of antibody for very high concentrations of Desmopressin present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Desmopressin concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Desmopressin.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C

2. Pipette **50 ul** of **prepared Standards** or **diluted Samples** into the respective wells.

3. Add **50 ul** of working **Biotinylated DDAVP** into the respective wells.

4. Cover the plate and incubate for **150 minutes** at 37°C

5. Aspirate and wash plate 4 times with **(1X) Wash Buffer with 1 minute soak time** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

6. Pipette without delay in the same order **100 ul** of working **Streptavidin:HRP Conjugate** into each well.

7. Cover the plate and incubate for **150 minutes** at **37°C**

8. Aspirate and wash plate 5 times with **Wash Buffer (1X) with 1 minute soak time** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

9. Add **100 ul** of **TMB Substrate** in each well.

10. Incubate the plate at **37°C** for **30 minutes** in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

11. Pipette out **50 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

12. Read the absorbance at 450 nm with a microplate reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis.

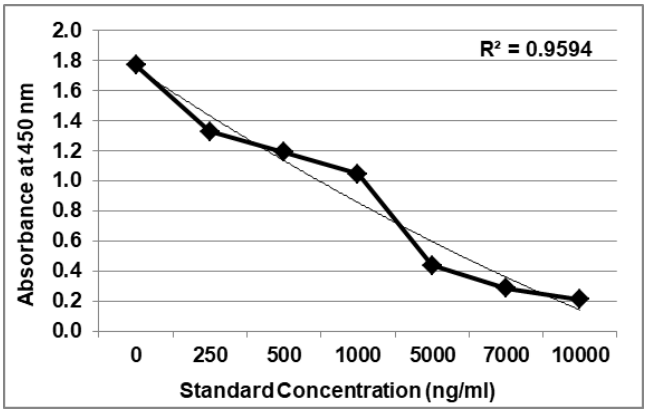
Draw the best fit curve through the standard points. To determine the unknown Desmopressin concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Desmopressin Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 10,000 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	1.772	--	--
250	1.330	275.3	110.1
500	1.191	514.4	102.9
1000	1.047	880.6	88.1
5000	0.439	5136.5	102.7
7000	0.287	7630.9	109.0
10000	0.212	9280.1	92.8

Typical Graph



Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Desmopressin present in matrix. The standard has been calibrated and validated against commercially sourced Minirin tablets[1].

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=3 assays) reproducibility on two pools with low (250 ng/ml), medium (5000 ng/ml) and high (10000 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<12%	<15%
Medium	<10%	<12%
High	<10%	<12%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

[1] **Minirin, Nocdurna, DDAVP is a registered trademark of Ferring Pharmaceuticals Inc.**

References:

The effect of tourniquet application, tranexamic acid, and desmopressin on the procoagulant and fibrinolytic systems during total knee replacement
MH Ellis, B Fredman, E Zohar, N Ifrach... - Journal of clinical ..., 2001 - Elsevier

Von Willebrand's disease: use of collagen binding assay provides potential improvement to laboratory monitoring of desmopressin (DDAVP) therapy
EJ Favaloro, M Dean, L Grispo... - American journal of ..., 1994 - Wiley Online Library

Desmopressin therapy to assist the functional identification and characterisation of von Willebrand disease: differential utility from combining two (VWF: CB and VWF ...
EJ Favaloro, J Thom, D Patterson, S Just, T Dixon... - Thrombosis research, 2009 - Elsevier

Pre-treatment of porcine pulmonary xenograft with desmopressin: a novel strategy to attenuate platelet activation and systemic intravascular coagulation in an ex-vivo ...
YT Kim, HJ Lee, SW Lee, JY Kim, HC Wi... - ..., 2008 - Wiley Online Library

[HTML] Laboratory diagnosis and monitoring of desmopressin treatment of von Willebrand's disease by flow cytometry
S Giannini, AM Mezzasoma, M Leone... - ..., 2007 - haematologica.org

Plasmin generation and fibrin (ogen) olysis following desmopressin infusion
H Takahashi, W Tatewaki, K Wada... - American journal of ..., 1991 - Wiley Online Library

Desmopressin: therapeutic limitations in children and adults with inherited coagulation disorders
B Nolan, B White, J . Smith, C O'reily... - British journal of ..., 2000 - Wiley Online Library

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Desmopressin equivalent
1A 2A	zero std zero std			
1B 2B	250 ng/ml 250 ng/ml			
1C 2C	500 ng/ml 500 ng/ml			
1D 2D	1000 ng/ml 1000 ng/ml			
1E 2E	5000 ng/ml 5000 ng/ml			
1F 2F	7000 ng/ml 7000 ng/ml			
1G 2G	10000 ng/ml 10000 ng/ml			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2022

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |