Human Anti-Diuretic Hormone/Vasopressin/Arginine Vasopressin (ADH/VP/AVP) GENLISA™ ELISA

REF : KBD1049

Ver 1.0

IVD

ELISA immunoassay for Quantitative Determination of ADH/VP/AVP in human serum and plasma.

IVD	**For In-vitro Diagnostic Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBD1049 Σ 96 tests

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Introduction:

Anti-diuretic hormone is produced by special nerve cells found at the base of the brain known as the hypothalamus. The nerve cells transport the hormone down the nerve fibres (axons) to the posterior pituitary gland and the hormone is released into the bloodstream. Anti-diuretic hormone helps to control blood pressure by acting on the kidneys and the blood vessels. The important role is to conserve the fluid volume of the body by reducing the amount of water passed out in the urine. It functions by allowing water in the urine to be taken back into the body in a specific area of the kidney. Thus, more water returns to the bloodstream, urine concentration rises and water loss is reduced. Higher concentrations of anti-diuretic hormone cause blood vessels to constrict (become narrower) and thus increases blood pressure. A deficiency of body fluid (dehydration) can only be finally restored by increasing water intake.

Intended Use:

The Human Anti-Diuretic Hormone/Vasopressin/Arginine Vasopressin (ADH/VP/AVP) GENLISA™ ELISA is intended for the quantitative determination of Anti-Diuretic Hormone/Vasopressin/Arginine Vasopressin (ADH/VP/AVP) in human serum and plasma.

Principle:

Human Anti-Diuretic Hormone/Vasopressin/Arginine Vasopressin (ADH/VP/AVP) GENLISA™ ELISA method employs sandwich enzyme linked immunosorbent assay (ELISA) technique. Antibodies are pre-coated onto microwells. Standards/Samples are pipetted into microwells and ADH/VP/AVP present in the sample are bound by the antibodies. Enzyme conjugate antibody is added and followed by Streptavidin-HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (A and B) is added to microwells and color develops proportionally to the amount of ADH/VP/AVP present in sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (96 wells) - 1 no
2. Standards (0.5 ml) (concentrated) - 480 pg/ml
3. Standard Diluent - 3 ml
4. Enzyme Conjugate - 1 ml
5. Streptavidin:HRP Conjugate - 6 ml
6. (40X) Wash Buffer (40X) - 20 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

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. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. 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. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5ml of Wash Buffer (40X) to 97.5ml of DI water. This is the working solution.
2. Standard Working Solution: Prepare the Standard Working Solution as per the table given below using the provided Standard Concentration and Standard Diluent.

Standard Concentration	Standard No	Dilution Particulars
480 pg/ml	Standard, concentrated	Original Standard provided in the Kit
240 pg/ml	Standard No.5	120 ul Original Standard + 120 ul Standard Diluent
120 pg/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
60 pg/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
30 pg/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
15 pg/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent

** refer accompanying sheet with the Standard, concentrated in the kit*

3. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **50 ul Standard Working Solution** in appropriate wells.
3. Add **40 ul Sample** into appropriate wells.
4. Add **10ul Enzyme Conjugate** to each sample well.
   Do not pipette into the blank and standards wells. The standards offered in the kit are pre-offered as a complex of the standard and the biotin antibody for ease-of-use
5. Add **50 ul Streptavidin:HRP Conjugate** into each well except blank well.
6. Cover the plate and incubate for 1 hour at 37°C in the incubator.
7. Aspirate and wash plate 5 times with **(1X) Wash Buffer** 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. All the washes should be performed similarly.
8. Add **100 ul** of **TMB Substrate** into each well.
9. Incubate at 37ºC for 10 minutes in dark.

10. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Using graph paper, plot the average value (absorbance 450 nm) 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 ADH/VP/AVP 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 ADH/VP/AVP Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) 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 sample absorbance is greater than highest standard, dilute with Standard 0 and re-assay.

Positive Results:

If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD.
10 replicates of '0' standards were evaluated and the LOD was found to be 1.285 pg/ml.

Specificity:
The antibodies used in this kit are specific to ADH/VP/AVP.

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Safety Precautions:

- **This kit is For In-vitro Diagnostic 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.

LIMITED WARRANTY

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **50 ul Standard Working Solution** in appropriate wells
3	Add **40 ul Sample** in appropriate wells
4	Add **10 ul Enzyme Conjugate** to each well except blank well.
5	Add **50 ul Streptavidin:HRP Conjugate** to each well except blank well.
6	Cover the plate and incubate for 1 hour at 37°C in the incubator.
7	Aspirate and **wash plate 5 times** with **(1X) Wash Buffer** 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. All the washes should be performed similarly.
8	Add **100 ul** of **TMB Substrate** into each well.
9	**Incubate** at **37ºC** for **10 minutes**.
10	Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (8X12 wells)
STD	Standard
STD DIL	Standard Diluent
ENZY CONJ	Enzyme Conjugate
STP HRP	Streptavidin:HRP
40X WASH BUF	(40X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature