Product Manual

Revision date: 23/Apr/2018

1-Desamino 8D Arginine Vasopressin (DDAVP) ELISA Kit

Catalog No.: abx150333

Size: 96T

Range: 123.46 pg/ml - 10000 pg/ml

Sensitivity: < 39.5 pg/ml

Storage: Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of DDAVP in Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: Desmopressin is a medication used to treat diabetes insipidus, bedwetting, hemophilia A, von Willebrand disease, and high blood urea levels. In hemophilia A and von Willebrand disease, it should only be used for mild to moderate cases. It may be given in the nose, by injection into a vein, by mouth, or under the tongue. Desmopressin works by limiting the amount of water that is eliminated in the urine; that is, it is an antidiuretic. It works at the level of the renal collecting duct by binding to V2 receptors, which signal for the translocation of aquaporin channels via cytosolic vesicles to the apical membrane of the collecting duct. The presence of these aquaporin channels in the distal nephron causes increasing water reabsorption from the urine, which becomes passively re-distributed from the nephron to systemic circulation by way of basolateral membrane channels. Desmopressin also stimulates release of von Willebrand factor from endothelial cells by acting on the V2 receptor.

Principle of the Assay

This kit is based on a competitive binding enzyme-linked immuno-sorbent assay technology. An antibody specific to DDAVP is pre-coated onto the 96 well plate. A competitive inhibition reaction is launched between biotin labeled DDAVP and unlabeled DDAVP with the pre-coated antibody specific to DDAVP. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain DDAVP will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is inverse proportional to the DDAVP amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of DDAVP can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Diluent A: 12 ml
6. Diluent B: 12 ml
7. Stop solution: 6 ml
8. TMB substrate : 9 ml
9. Detection Reagent A (100X): 120 µl
10. Detection Reagent B (100X): 120 µl
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. Tubes to prepare standard or sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

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Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2).

» If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.

» Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

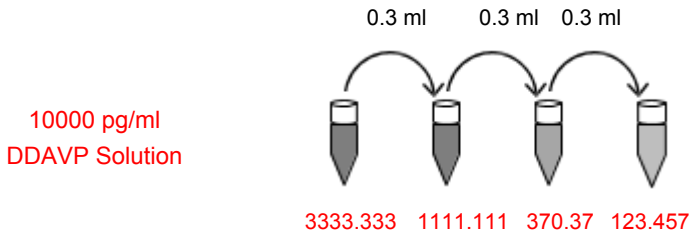
» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Bring samples and all kit components to room temperature. Prepare the Standard with 0.5 ml of Standard Diluent buffer (kept for 10 min at room temperature) to make the 10000 pg/ml Standard Solution. Allow the reconstituted standard to sit for 10 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles. Label 4 tubes with 3333.33 pg/ml, 1111.11 pg/ml, 370.37 pg/ml, 123.46 pg/ml. Aliquot 0.6 ml of the Standard diluent buffer into each tube. Add 0.3 ml of 10000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube, mix thoroughly, and so on.



4. Detection Reagent A and B Preparation

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Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with the Diluent A and B respectively and mixed thoroughly. They are sticky solutions, therefore pipette with a slow, smooth action to reduce volume errors. The solution should be prepared no more than 2 hours prior to the experiment. Please discard after use.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate or triplicate.
2. Add 50 µl of the diluted standards into the standard wells. Aliquot 50 µl Standard Diluent Buffer to the control (zero) well.
3. Add 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Immediately aliquot 50 µl of Detection Reagent A working solution (if it appears cloudy mix gently until the solution is uniform) to each well. Shake the plate gently to mix thoroughly (a microplate shaker is recommended).
5. Seal the plate with a cover and incubate for 1 h at 37°C.
6. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
7. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
8. Repeat the aspiration/wash process 5 times as explained in step 6.
9. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
10. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between DDAVP concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

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C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. It is recommended measuring each standard and sample in duplicate or triplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.
10. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of DDAVP were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of DDAVP were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

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