Product Manual

Revision date: 12/Jul/2018

Human Vesicular Monoamine Transporter 2 (VMAT2) CLIA Kit

Catalog No.: abx494600

Size: 96T

Range: 0.014 ng/ml - 10 ng/ml

Sensitivity: < 0.006 ng/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 Vesicular Monoamine Transporter 2 (VMAT2) in Human Tissue Homogenates, Cell Lysates and other biological fluids.

Introduction: Vesicular monoamine transporter 2 (VMAT2) also known as solute carrier family 18 member 2 (SLC18A2) is an integral membrane protein that transports monoamines—particularly neurotransmitters such as dopamine, norepinephrine, serotonin, and histamine—from cellular cytosol into synaptic vesicles. In nigrostriatal pathway and mesolimbic pathway dopamine-releasing neurons, VMAT2 function is also necessary for the vesicular release of the neurotransmitter GABA.

Principle of the Assay

This kit is based on chemiluminescent immunoassay technology. Anti-Vesicular Monoamine Transporter 2 (VMAT2) antibody is pre-coated onto a 96-well plate. The standards and samples are added to the wells with a biotin conjugated anti-Vesicular Monoamine Transporter 2 (VMAT2) and incubated. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After the mixture of substrate A and B is added only wells that contain Vesicular Monoamine Transporter 2 (VMAT2) will produce chemiluminescence. The intensity of the emitted light is proportional to the amount of Vesicular Monoamine Transporter 2 (VMAT2) in the sample or standard.

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. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent A: 12 ml
8. Diluent B: 12 ml
9. Substrate A: 10 ml
10. Substrate B: 2 ml
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Luminometer capable of reading 96-well microplates (lag time 30.0 secs and read time 1.0 sec/well)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. Distilled or deionized water
6. Tubes to prepare standard or sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 liter graduated cylinders

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.

- **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.
- **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.
- **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.

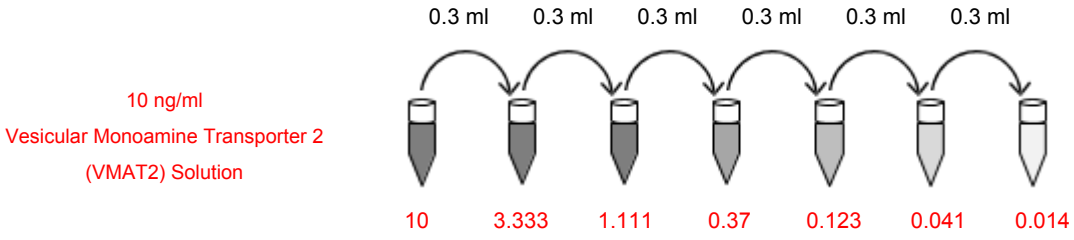
» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

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 1 ml of Standard Diluent buffer to make the 10 ng/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 6 tubes with 3.33 ng/ml, 1.11 ng/ml, 0.37 ng/ml, 0.12 ng/ml, 0.04 ng/ml, and 0.01 ng/ml respectively. Aliquot 0.6 ml of the Standard diluent buffer into each tube. Add 0.3 ml of 10 ng/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

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 and mixed thoroughly. They are sticky solutions, therefore pipette with a slow, smooth action to reduce volume errors. Please discard after use.

5. Substrate working solution Preparation

Substrate A and B should be mixed with a ration of 99:1 respectively and mixed thoroughly. For example, prepare 1 ml of substrate working solution by mixing 0.99 ml of Substrate A and 0.01 ml of Substrate B.

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 respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Add the solution at the bottom of each well without touching the side walls.
2. Add 100 µl of the diluted standards into the standard wells. Aliquot 100 µl Standard Diluent Buffer to the control (zero) well.
3. Add 100 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. Seal the plate with a cover and incubate for 1 h at 37°C.
5. Remove the cover and discard the liquid. Do not wash.
6. Aliquot 100 µl of the detection Reagent A working solution to each well. Seal the plate with a cover and incubate for 1 h at 37°C.
7. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350µL) 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.
8. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
9. Discard the solution and wash the plate 5 times with wash buffer as explained in step 7 (each time let the Wash Buffer stay for 1-2 min).
10. Aliquot 100 µl of Substrate working solution into each well. Seal the plate with a cover and incubate at 37°C for 10 min.
11. Measure the chemiluminescence signal in a microplate luminometer immediately.

For calculation, (the relative RLU) = (the RLU of each well) – (the RLU of Zero well). The standard curve can be plotted as the relative RLU of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Vesicular Monoamine Transporter 2 (VMAT2) concentration of the samples can be interpolated from the standard curve.

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

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 dilutions 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.
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 use expired components or components from a different kit.
10. Store the substrate A and B in the dark.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of Vesicular Monoamine Transporter 2 (VMAT2) were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of Vesicular Monoamine Transporter 2 (VMAT2) were tested on 3 different plates, 8 replicates in each plate.

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

Intra-Assay: CV<10%

Inter-Assay: CV<12%