Human GDI1 ELISA Kit

Catalog No.: abx151647

Size: 96T

Range: 0.156 ng/ml - 10 ng/ml

Sensitivity: < 0.052 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 GDP Dissociation Inhibitor 1 (GDI1) in Human Tissue Homogenates and other biological fluids.

Introduction: GDP dissociation inhibitors are proteins that regulate the GDP-GTP exchange reaction of members of the rab family, small GTP-binding proteins of the ras superfamily, that are involved in vesicular trafficking of molecules between cellular organelles. GDIs slow the rate of dissociation of GDP from rab proteins and release GDP from membrane-bound rabs. GDI1 is expressed primarily in neural and sensory tissues. Mutations in GDI1 have been linked to X-linked nonspecific mental retardation.

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to GDP Dissociation Inhibitor 1 (GDI1) is pre-coated onto a 96-well plate. The standards and samples are added to the wells and incubated. Biotin conjugated anti-GDP Dissociation Inhibitor 1 (GDI1) antibody is used as detection antibody. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain GDP Dissociation Inhibitor 1 (GDI1), biotin-conjugated antibody and enzyme-conjugated Avidin will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the GDP Dissociation Inhibitor 1 (GDI1) amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of GDP Dissociation Inhibitor 1 (GDI1) can be calculated.

Kit components

1. One pre-coated 96 well plate
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. Stop solution: 6 ml
10. TMB substrate : 9 ml
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume 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.
- **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.
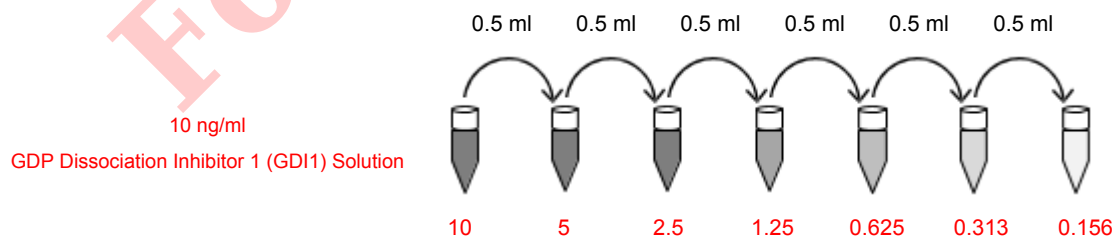
Note:

» 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 (kept for 10 min at room temperature) to make the 10 ng/ml Standard Solution (use within one hour), which serves as the highest standard. Mix gently and avoid foaming or bubbles. Label 6 tubes with 5 ng/ml, ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.63 ng/ml, 0.31 ng/ml and 0.16 ng/ml respectively. Aliquot 0.5 ml of the Standard diluent buffer into each tube. Add 0.5 ml of 10 ng/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from 1st tube to 2nd tube, mix thoroughly, and so on.

10 ng/ml
GDP Dissociation Inhibitor 1 (GDI1) Solution

| 0.5 ml | 0.5 ml | 0.5 ml | 0.5 ml | 0.5 ml | 0.5 ml |

| 10 | 5 | 2.5 | 1.25 | 0.625 | 0.313 | 0.156 |

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. The solution should be prepared no more than 2 hours prior to the experiment. Prepared Standard, Detection Reagent A and B can only be used once.

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.

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. Discard the solution and wash the plate 3 times with wash buffer. Do not let the wells completely dry at any time.

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer and incubate on an ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure three times.

Automated Washing: Discard the solution and wash the plate three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

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 (each time let the Wash Buffer stay for 1-2 min). Discard the Wash Buffer and blot the plate onto paper towels or other absorbent material.

10. 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.

11. 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.

12. 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.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The GDP Dissociation Inhibitor 1 (GDI1) 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 standards within 15 min of starting the experiment. 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. 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 TMB substrate in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrate for 30 min at room temperature. 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 GDP Dissociation Inhibitor 1 (GDI1) were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of GDP Dissociation Inhibitor 1 (GDI1) were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%