Product Manual

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Human Anti-Beta-1-Adrenergic Receptor Autoantibody (B1AR-Ab) ELISA Kit

Catalog No.: abx392291

Size: 96T

Range: 0.781 ng/ml - 50 ng/ml

Sensitivity: < 0.47 ng/ml

Storage: Store the 96-well plate and standards at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of B1AR-Ab in Human Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: The adrenergic receptors are a class of cell membrane-bound protein receptors throughout the body that are targets of the catecholamines, especially norepinephrine and epinephrine. The binding to these receptors by catecholamines will generally stimulate the sympathetic nervous system, the arm of the autonomic nervous system responsible for the fight-or-flight response.

Principle of the Assay

This kit is based on indirect enzyme-linked immuno-sorbent assay technology. The 96-well plate is pre-coated with the target antigen. The standards and samples are added to the wells and incubated. Biotin conjugated antibody is used as the detection antibody. Streptavidin-HRP is added to each well, incubated and then unbound conjugates are washed away using wash buffer. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the B1AR-Ab amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of B1AR-Ab can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample/Standard diluent buffer: 20 ml
4. Biotin conjugated antibody (Concentrated): 120 µl
5. Antibody diluent buffer: 10 ml
6. Streptavidin-HRP Conjugate (Concentrated): 120 µl
7. HRP Diluent Buffer: 10 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml
11. Plate Sealer: 5

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. Deionized or distilled water
7. 1.5 ml tubes to prepare standard/sample dilutions
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated 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 EDTA-Na_2 as an anticoagulant. Centrifuge for 15 minutes at 1000 x 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:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

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

General Sample guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (500 ng/ml - 5000 ng/ml), dilute 1:100, for medium concentration (50 ng/ml - 500 ng/ml), dilute 1:10 and for low concentration (0.781 ng/ml - 50 ng/ml), dilute 1:2. Very low concentrations (≤ 0.781 ng/ml) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

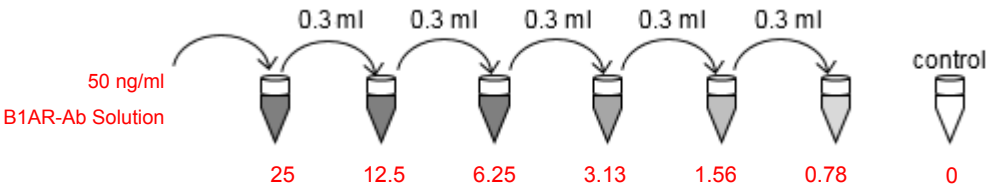
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Preparation of the B1AR-Ab standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for one minute.

a.) 50 ng/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube, keep the tube at room temperature for 10 min, mix gently (do not vortex!) and avoid foaming or bubbles. Use within one hour.

b.) 25 ng/ml → 0.78125 ng/ml standard solutions: Label 6 tubes with 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml, 1.5625 ng/ml and 0.78125 ng/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 50 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: 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. Use the diluted Standards for a single assay procedure and discard after use.

4. Preparation of Streptavidin-HRP Conjugate working solution: prepare no more than 30 min. before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Streptavidin-HRP Conjugate with HRP diluent buffe at 1/100 and mix thoroughly. i.e. Add 1 µl of Streptavidin-HRP Conjugate into 99 µl of HRP diluent buffer.

5. Preparation of Biotin conjugated antibody working solution: prepare no more than 30 min. before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of antibody diluent buffer.

B. Assay Procedure
Equilibrate the TMB substrate at 37°C, for at least 30 minutes prior to use. It is recommended to plot a standard curve for each test.

1. **Wash the plate two times before adding standard, samples and buffers.** Any strips that are not being used should be kept dry and stored at 4°C. 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. Add the solution at the bottom of each well without touching the side walls. Mix the standards and samples up and down to be homogeneous before adding into the wells but avoid adding bubbles.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37 °C for 90 minutes.
6. Remove the cover and discard the liquid. Wash the plate two times with wash buffer.
7. Add 100 µl of prepared Biotin conjugated antibody working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
8. 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.
9. Add 100 µl of HRP detection antibody working solution into each well, cover the plate and incubate at 37 °C for 30 minutes.
10. Remove the cover and wash the plate 5 times with Wash buffer as explained in step 8.
11. Add 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 only, the optimal time should be determined by end user. Do not exceed 30 min.

12. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.

13. 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 Human B1AR-Ab 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.

3. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

4. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.

6. 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.

7. Ensure plates are properly sealed or covered during incubation steps.

8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.

9. To avoid cross contamination do not reuse pipette tips and tubes.

10. Do not use components from a different kit or expired ones.

11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The 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 B1AR-Ab were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of B1AR-Ab were tested on 3 different plates, 8 replicates in each plate.

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

Intra-Assay: CV<8%

Inter-Assay: CV<10%

E. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Concentration ng/ml	0	0.78125	1.5625	3.125	6.25	12.5	25	50
OD450	0.121	0.176	0.234	0.318	0.513	1.064	1.484	2.454