Mouse Anti-Acetylcholine Receptor Antibody

AntI-AChR ELISA Kit

Cat #: orb1176642 (manual)

The kit is designed to qualitatively determine mouse mAchR in serum and plasma.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid from bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all testing samples are tested in duplicate.
- If the blue color develops too shallow after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time (Do not over develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.

Intended use

The kit is used to detect the existence or not of mouse mAchR in serum or plasma.

Assay time: 90 min

Validity: six months

Store at: 2-8 °C

Assay principle

The kit uses a sandwich enzyme-linked immunosorbent assay (ELISA) to detect the existence or not of mouse mAchR in samples. Test samples are added into wells pre-coated with recombinant mouse muscarinic AchR proteins and incubated appropriately. Following washing to remove unbound antibody and other serum components in the wells, add the HRP-conjugated anti- mouse IgG to bind the analyte, followed by incubation and washing procedures. Finally, HRP substrates are added, incubated for detection, and a blue color is developed. Reaction is stopped and color turns to yellow when Stopping

Solution (acidic) is added. The existence or not of mouse mAchR in the samples is determined by comparing the OD of the samples to the CUTOFF value.

Materials supplied

Microelisa Stripplate precoated with mouse mAchR	1x96 well
Negative Control	0.35 ml X 1 vial
Positive Control	0.35 ml X 1 vial
Sample diluent	30mlx1
10 X Wash Solution	100 ml
HRP-Conjugate Reagent	12 ml
TMB developing reagent	6 ml
Stop Solution	6 ml
Closure plate membrane	2
Sealed bag	1
Package insert	1

Materials required but not supplied

- 37°C incubator.
- Standard microplate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

NOTE: Serum or plasma to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤2months) or -80°C (≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.

Sample Preparation

- Biorbyt is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- Wash Solution - dilute 1 part concentrate (10x) + 9 parts deionized or distilled water.

Assay procedures

1. Prepare all the controls and samples before starting assay procedure (Please read Reagents Preparation). It is recommended that all controls and samples should be added in duplicate to the Microtiter Plate.

2. Set Negative control, Positive control and test sample wells on the assay plate. Allow six Control determinations (two Blanks, two Negative Controls, and two Positive Controls) per run.

3. Add 100 µ l of each control and samples to corresponding wells. Cover and incubate the plate for 30 minutes at 37°C.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

Automated Washing: Wash plate FIVE times with diluted wash solution (350- 400µl/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

5. Add 100µl of HRP-conjugated antibody to each well. Mix well. Mixing well in this step is important. Cover and incubate the plate for 30 minutes at 37°C.

6. Wash the plate as described in step 4.

7. Add 50µl TMB to each well. Cover and incubate for 10-30 minutes at 37°C (Protect from light. Do not over develop).

8. Add 50µl Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microplate reader immediately.

Determine the Result

Test validity

The average of Positive control wells ⩾ 1.00; the average of Negative control wells ⩽ 0.15.

Interpretations of the results

Calculate Critical (CUTOFF): = The average of Negative control wells + 0.15

Negative result: Sample OD < Calculate Critical (CUTOFF) is Negative

Positive result: Sample OD ⩾ Calculate Critical (CUTOFF) is Positive

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