ab287812 – QuickDetect Mouse Acetylcholine ELISA Kit

For the quantitative *in vitro* determination of Acetylcholine
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287812

Storage and Stability

The entire kit may be stored at 4°C in the dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Strip-plate	1 Unit	4°C
Standard 180 pg/mL	1 x 0.5 mL	4°C
Standard diluent	1 x 6 mL	4°C
HRP-Conjugate Reagent	10 mL	4°C
Sample Diluent	6 mL	4°C
Chromogen Solution A	6 mL	4°C
Chromogen Solution B	6 mL	4°C
Stop Solution	6 mL	4°C
20X Wash buffer	25 mL	4°C
Closure plate membrane	2 units	4°C
Sealed bags	1 unit	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Micro ELISA Strip-plate, reseal them in zip-lock foil and keep at 4°C.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Δ Note: *Note: Standard concentration was followed by: 800, 400, 200,100, 50, 25 pg/ml*

Wash Buffer: Dilute the concentrated washing buffer (20X) with distilled water.

Δ Note *Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.*

Version 4, Last updated May 20, 2024

Sample Preparation

Δ Note: *Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay can not be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with NaN_3 should be avoided for this assay.*

Serum: After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.

Plasma: Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuged again

Urine: Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine samples.

Cell Samples: If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/mL with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again.

Tissue Samples: Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Δ Note: *End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.*

Standard Prepation

- Ten wells are set for standards in a Micro ELISA strip-plate.

1. In Well 1 and Well 2, 50µl Standard solution and 50µl Standard Dilution buffer are added and mixed well.
2. In Well 3 and Well 4, 50µl solution from Well 1 and Well 2 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 3 and Well 4.
3. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well.

4. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well.
5. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 9 and Well 10.
6. After dilution, the total volume in all the wells are 50µl and the concentrations are 90pg/ml ,45pg/ml, 22.5pg/ml, 11.25pg/ml, and 5.625pg/ml, respectively.

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run for each assay.

1. Prepare all reagents, samples, and standards as instructed above.
2. In sample wells, add 40 µL Sample dilution buffer and 10 µL samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Add HRP-Conjugate reagent 100 µL to each well, except blank well.
4. Incubate for 60 min at 37°C after sealed with Closure plate membrane.
5. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times, dry by pat.
6. Add 50 µL Chromogen Solution A and 50 µL Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.
7. Add 50 µL stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
8. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

Calculations

Known concentrations of Mouse ACh Standard and its corresponding reading OD is plotted respectively. The concentration of Mouse ACh in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

If the OD value of the sample is greater than the first well of standards, please dilute the sample (n times) before test. When calculating the original AFU concentration, please multiply the total dilution factor (Xn).

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

Version 4, Last updated May 20, 2024

www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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Storage and Stability

The entire kit may be stored at 4°C in the dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Strip-plate	1 Unit	4°C
Pre-diluted standard*	6 x 0.3 mL	4°C
HRP-Conjugate Reagent	10 mL	4°C
Sample Diluent	6 mL	4°C
Chromogen Solution A	6 mL	4°C
Chromogen Solution B	6 mL	4°C
Stop Solution	6 mL	4°C
20X Wash buffer	25 mL	4°C
Plate sealers	2 units	4°C

*** Prediluted standard concentrations are as follow: 800 pg/ml, 400 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml**

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from the Mouse ACH Antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Version 4, Last updated May 20, 2024

Wash Buffer: Dilute the concentrated washing buffer (20X) with distilled water.

Δ Note *Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.*

Sample Preparation

Δ Note: *Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay can not be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with NaN_3 should be avoided for this assay.*

Serum: After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again.

Plasma: Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuged again

Urine: Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine samples.

Cell Samples: If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X1,000,000/mL with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again.

Tissue Samples: Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Δ Note: *End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.*

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.

- A standard curve must be run for each assay.

1. Prepare all reagents, and samples as instructed in the previous section
2. Add 50 µL standard or samples to the appropriate wells. Leave a well empty as blank control.
3. Add 100 µL of HRP-Conjugate reagent to each well, except the blank well. Cover the plate with the plate sealer and incubate for 60 min at 37°C.
4. Wash the plate 4 times:
   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry the plate with 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.
   Automated Washing: Aspirate all wells, then wash plates four times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µL/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
5. Add 50 µL Chromogen Solution A and 50 µL Chromogen Solution B to each well, mix gently and incubate at 37°C for 15 minutes in the dark.
6. Add 50 µL stop solution to each well. The color in the well should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Read the absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.
8.

Calculations

Known concentrations of Mouse ACh Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Mouse ACh in sample is determined by plotting the sample's O.D. on the X-axis. The original concentration is calculated by multiplying the dilution factor.

If the OD value of the sample is greater than the first well of standards, please dilute the sample (n times) before test. When calculating the original AFU concentration, please multiply the total dilution factor (Xn).

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