ab287811 – QuickDetect Human 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/ab287811

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	4°C
Standard (225 pg/mL)	0.5 mL	4°C
Standard Diluent	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
Plate sealers	2 units	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.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

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

Standard Preparation

- Ten wells are set for standards in a Microelisa stripplate.
- In Well 1 and Well 2, 50 µL Standard solution and 50 µL Standard Dilution buffer are both added and mixed in each well.
- In Well 3 and Well 4, 50 µL solution from Well 1 and Well 2 are added respectively. Then 50 µL Standard Dilution buffer are added and mixed well. 50 µL solution is discarded from Well 3 and Well 4.
- In Well 5 and Well 6, 50 µL solution from Well 3 and Well 4 are added respectively. Then 50 µL Standard Dilution buffer are added and mixed well.
- In Well 7 and Well 8, 50 µL solution from Well 5 and Well 6 are added respectively. Then 50 µL Standard Dilution buffer are added and mixed well.
- In Well 9 and Well 10, 50 µL solution from Well 7 and Well 8 are added respectively. Then 50 µL Standard Dilution buffer are added and mixed well.
- 50 µL solution is discarded from Well 9 and Well 10.
- After dilution, the total volume in all the wells are 50 µL and the concentrations are 112.5 pg/ml, 56.25 pg/ml, 28.13 pg/ml, 14.06 pg/ml and 7.03 pg/ml, respectively

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.*

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 in the previous section
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 60 min at 37°C after sealed with plate sealer.
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.
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 Human ACh Standard and its corresponding reading OD is plotted respectively. The concentration of Human 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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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	Room Temperature
Pre-diluted standard*	0.3 mL x 6	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	Room Temperature

* Prediluted standard concentrations are as follow: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 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.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

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

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 as it can inhibit activity of HRP.*

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.

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- Remove any unneeded strips from Human ACH Antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run for each assay.
- Samples should be added to the Microplate in less than 5 minutes.

1. Prepare all reagents, samples and standards before starting assay procedure
2. Add 50 µL standard or sample into the appropriate wells. Leave a well empty as blank control.
3. Add 100 µL of HRP-Conjugate reagent to each well, except blank well. Cover with a plate sealer and incubate 60 min at 37°C.
4. Wash the Microtiter 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 by hitting 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.
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 Human 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 Human 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.

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