



Human ACHE ELISA Kit

Enzyme Immunoassay for the quantification of Human ACHE in Human Serum, plasma (heparin, EDTA) and cell culture supernatants.

Catalog number: ARG83648

Package: 96 wells

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INTRODUCTION

Acetylcholinesterase hydrolyzes the neurotransmitter, acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. It is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen. Acetylcholinesterase exists in multiple molecular forms which possess similar catalytic properties, but differ in their oligomeric assembly and mode of cell attachment to the cell surface. It is encoded by the single ACHE gene, and the structural diversity in the gene products arises from alternative mRNA splicing, and post-translational associations of catalytic and structural subunits. The major form of acetylcholinesterase found in brain, muscle and other tissues is the hydrophilic species, which forms disulfide-linked oligomers with collagenous, or lipid-containing structural subunits. The other, alternatively spliced form, expressed primarily in the erythroid tissues, differs at the C-terminal end, and contains a cleavable hydrophobic peptide with a GPI-anchor site. It associates with the membranes through the phosphoinositide (PI) moieties added post-translationally. AChE activity may constitute a sensitive biomarker of RBC ageing in vivo, and thus, may be of aid in understanding the effects of transfusion[provided by RefSeq, Sep 2019]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Human ACHE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Human ACHE present is bound by the immobilized antibody. After washing away any

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unbound substances, a biotin-conjugated antibody specific for Human ACHE is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Human ACHE bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm \pm 2nm. The concentration of Human ACHE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8x 12 strips	4°C.
Standard (Lyophilized)	2x 10 ng/tube	4°C
Standard/Sample diluent	30 ml (Ready to use)	4°C
Antibody conjugate concentrate (100X)	1 vial (100 µl)	4°C
Antibody diluent buffer	12 ml (Ready to use)	4°C
HRP-Streptavidin concentrate (100X)	1 vial (100 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
25X Wash Buffer	20 ml	4°C
TMB substrate	10 ml (Ready to use)	4°C (Protect from light)
STOP solution	10 ml (Ready to use)	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. The kit can also be stored at -20°C for long-term storage.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- If crystals are observed in the 25X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for few minutes before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1000 x g and aliquot & store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **25 X wash buffer** with **deionized water** to yield 1X wash buffer. The diluted 1X wash buffer is stable for a week at 2°C to 8°C.
- **1X Antibody conjugate:** It is recommended to prepare this reagent immediately prior to use and use it within 2 hours after preparation. Dilute **100X antibody conjugate concentrate** into **Antibody diluent buffer** to yield 1X detection antibody solution. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- **1X HRP-Streptavidin Solution:** It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute **100X HRP-Streptavidin concentrate solution** into **HRP-Streptavidin diluent buffer** to yield 1X HRP-Streptavidin Solution buffer. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)
- **Sample:** If the initial assay found samples contain Human ACHE higher than the highest standard, the samples can be diluted with **Standard/Sample diluent** and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.
(It is recommended to do pre-test to determine the suitable dilution factor).

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- **Standards:** Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with **1 ml Standard/Sample diluent** to yield a stock concentration of 10000 pg/ml. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml.

Dilute Human ACHE standard as according to the table below:

Standard	Human ACHE Conc. (pg/ml)	Standard/Sample diluent (μl)	Standard (μl)
S7	4000 pg/ml	600	400 (<u>10000 pg/ml</u> Stock)
S6	2000 pg/ml	500	500 (S7)
S5	1000 pg/ml	500	500 (S6)
S4	500 pg/ml	500	500 (S5)
S3	250 pg/ml	500	500 (S4)
S2	125 pg/ml	500	500 (S3)
S1	62.5 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

The 1X HRP-Streptavidin Solution and TMB substrate must be kept warm at 37°C for 20-30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard Human ACHE detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of Human ACHE amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, samples** and **zero controls** (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes** at **37°C**.
3. Aspirate each well. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. Do NOT let the wells completely dry at any time. Wash step is not necessary in this step.
4. Add **100 µl 1X Antibody conjugate** into each well, gently tap the plate to mix well. Cover wells and incubate for **60 minutes** at **37°C**.
5. Aspirate each well and wash, repeating the process two times for a **total three washes**. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Do NOT let the wells completely dry at any time.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well, gently tap the plate to mix well. Cover wells and incubate for **30 minutes** at **37°C**.

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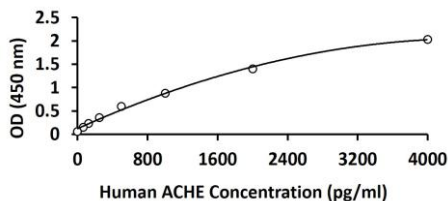
7. Aspirate each well and wash as step 5, but wash for a **total five washes**.
8. Add **90 µl** of **TMB substrate** to each well. Incubate for **25-30 minutes** at **37°C** in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated Human ACHE standard solutions; the other wells show no obvious color).
9. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at 450nm immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (<https://www.arigobio.com/elisa-analysis>)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human ACHE ranged from 62.5- 4000 pg/ml. The mean MDD was 15 pg/ml.

Specificity

This assay recognizes natural and recombinant Human ACHE. No significant cross-reactivity or interference with the factors below was observed:

Intra-assay and Inter-assay precision

The CV values of intra-assay was < 10% and inter-assay was < 10%.