Acetylcholinesterase Fluorescent Activity Kit

Catalog Number EIAACHEF (192 tests)

Pub. No. MAN0019411 **Rev** 4.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Acetylcholinesterase Fluorescent Activity Kit is a fluorescent activity assay designed to measure acetylcholinesterase activity in a variety of samples. The kit uses a proprietary non-fluorescent molecule to covalently bind the thiol product of the reaction between the substrate and acetylcholinesterase to produce a fluorescent product (390 nm excitation, 510 nm emission). The assay can be run as an end point assay, or as a kinetic activity assay.

This assay measures the activity of acetylcholinesterase in serum, plasma (EDTA and heparin), and erythrocyte membranes. The assay was validated with human acetylcholinesterase, but is expected to measure acetylcholinesterase activity in samples from other species, including chicken, mouse, rat, dog, monkey, and pig.

Acetylcholinesterase (AChE) is a neurotransmitter in the central and peripheral nervous systems. It is important in the development and function of the nervous system. Alternative splicing and post translational associations of catalytic and structural subunits results in a number of isoforms. The major form of acetylcholinesterase found in brain, muscle, and other tissues is the hydrophilic species. The other is expressed primarily in the erythroid tissues

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Acetylcholinesterase Standard; 1,000 mU/mL acetylcholinesterase in stabilizing solution	225 µL
Assay Buffer Concentrate (10X)	28 mL
Black 96-well Plate	2 plates
Detection Reagent; reconstitute with Dry DMSO	2 vials
Dry DMSO (dimethyl sulfoxide)	14 mL
AChE Substrate; Acetylthiocholine iodide	2 vials

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 390 nm
- Calibrated adjustable precision pipettes and plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Assay Buffer

1. Dilute 7 mL of Assay Buffer (10X) with 63 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare erythrocyte (RBC) membrane samples

1. Collect blood in the presence of heparin or EDTA.
2. Centrifuge the sample and remove the plasma and white cell layer from the erythrocyte (RBC) layer.
3. Suspend the RBCs and gently wash twice with three volumes of isotonic saline (0.9%).
4. Separate the cells by centrifugation at 600 × *g* for 10 minutes and discard the saline after each step.
5. Add four volumes of cold deionized water to the RBC sample and vortex to lyse cells.
6. Incubate sample for 10 minutes at 4°C, or perform a freeze-thaw.
7. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and discard the supernatant.
8. Wash the membrane pellet with two or three volumes of isotonic saline and centrifuge.
9. Repeat wash step until the pellet is only slightly pink. Do not disturb the smaller dark red pellet on the bottom (non-lysed RBCs).
10. Solubilize the white erythrocyte membrane (ghost) pellet with Triton™ X-100. Do not disturb the residual pellet of non-lysed RBCs.
11. Store samples on ice until assaying or freeze in aliquots for later use.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at on ice until ready to perform assay.

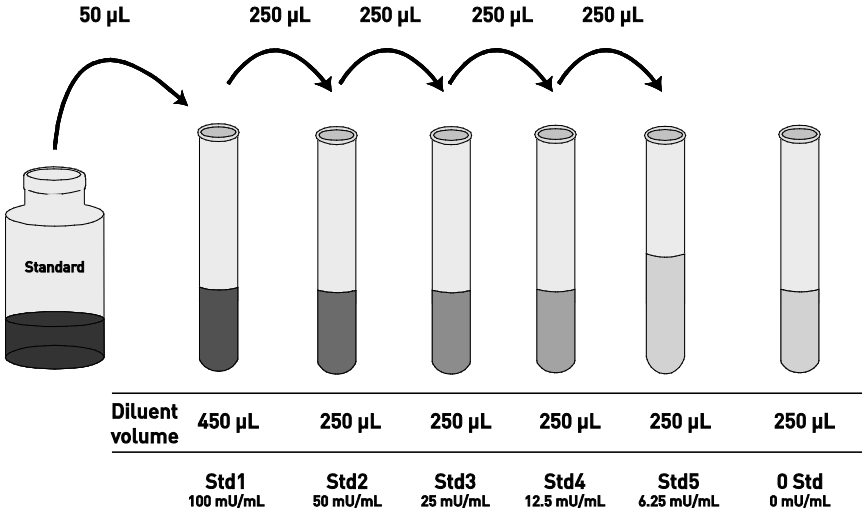
- Dilute **serum and plasma** samples ≥1:300 in 1X Assay Buffer.
- Dilute **erythrocyte membrane** samples so that the Triton™ X-100 content is ≤0.01% when performing the assay.

Dilute standards

Note: Use plastic tubes for diluting standards.

Note: One unit of AChE is defined as the amount of enzyme needed to hydrolyze 1.0 micromole of acetylthiocholine iodide per minute at 25°C.[1]

1. Add 50 µL Acetylcholinesterase Standard to one tube containing 450 1X Assay Buffer and label as 100 mU/mL acetylcholinesterase.
2. Add 250 µL 1X Assay Buffer to each of 5 tubes labeled as follows: 50, 25, 12.5, 6.25, and 0 mU/mL acetylcholinesterase.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Reconstitute Detection Reagent

Note: The Detection Reagent reacts with strong nucleophiles (e.g., buffers containing sodium azide, Proclin™, or Kathon™ preservatives).

1. Allow the Detection Reagent to reach room temperature in the sealed bag before opening.
2. Add 700 µL of the Dry DMSO to the vial of Detection Reagent and nutate for 5 minutes.
   Note: DMSO is an aprotic organic solvent shown to enhance the absorption rate of skin-permeable substances. Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.
3. The reconstituted Detection Reagent is a 10X concentrate. Store any unused reconstituted Detection Reagent at 4°C in the desiccated pouch. **Use within 2 months**.

[1] Järvinen, P et al. (2010) Miniaturization and Validation of the Ellman's Reaction Based Acetylcholinesterase Inhibitory Assay into 384-Well Plate Format and Screening of a Chemical Library. Combinatorial Chemistry & High Throughput Screening 278-284 (7).

Reconstitute AChE Substrate

1. Add 700 µL of the Dry DMSO to the vial of Ache Substrate and nutate for 5 minutes.
   Note: Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.
2. The reconstituted AChE Substrate is a 10X concentrate. Store any unused reconstituted AChE Substrate at room temperature for use **within 2 months**.

Prepare reaction mix

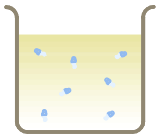
Prepare a reaction mix for detection of enzyme activity according to the table. Use the prepared Reaction Mix within 30 minutes.

Reagent	½ plate	Full plate
10X AChE Substrate Concentrate	300 µL	550 µL
10X Detection Reagent Concentrate	300 µL	550 µL
Dry DMSO	2.4 mL	4.4 mL
Total volume	3.0 mL	5.5 mL

Assay procedure

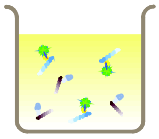
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 20 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample

Add 100 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add detection reagent and substrate

a. Add 50 µL of Reaction Mix to each well.
b. Shake the plate at room temperature for 20 minutes at 700 - 900 rpm.
c. Proceed to read the plate immediately.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 510 nm, with excitation at 390 nm.
2. Use curve-fitting software to generate the standard curve. A linear regression provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the activity of unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals greater than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the activity by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–100 mU/mL acetylcholinesterase.

Standard Acetylcholinesterase (mU/mL)	Mean FLU
100	51,499
50	29,926
25	14,753
12.5	8,543
6.25	5,496
0	2,379

Intra-assay precision

Three mammalian samples were assayed in replicates of 22 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mU/mL)	45.3	19.5	5.7
%CV	2.6	3.8	4.4

CV = Coefficient of Variation

Inter-assay precision

Three mammalian samples were assayed multiple times in duplicate by 5 operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mU/mL)	43.4	19.4	6.5
%CV	6.9	6.1	12.3

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

A variety of serum and plasma samples were tested with the assay. The average activity and activity ranges are given below.

Sample	Range (mU/mL)	Average (mU/mL)
Plasma	5,341 - 11,502	9,039
Serum	6,608 - 16,085	10,789

Interferents

A variety of solvents were tested as possible interfering substances in the assay.

- 5% ethanol in the well increased the activity by 6.4%, whereas 10% decreased activity by almost 43%.
- 5% DMSO or DMF in the well decreased activity by 12.6% and 19.7% respectively.
- 10% methanol in the well decreased activity by 6.3%.
- Solvent levels at 1% of well volume are expected to have little or no effect on the measured activity. A solvent only control should be run when appropriate.

Linearity of dilution

Linearity was determined using high and low concentration serum samples mixed in the ratios shown in the following table.

Low Sample %	High Sample %	Expected Conc. (mU/mL)	Observed Conc. (mU/mL)	% Recovery
80	20	21.2	20.4	96.4
60	40	35.4	33.1	93.5
40	60	49.7	50.2	101.0
20	80	63.9	62.9	98.4
			Mean Recovery	**97.3**

Sensitivity

The analytical sensitivity of the assay is 0.063 mU/mL acetylcholinesterase. This was determined by adding two standard deviations to the mean FLU obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

The assay was tested with a sample of human butyrylcholinesterase at 20 mU/mL, producing a reading 1.5 times higher than the same concentration of AChE tested at the same time.

Limited product warranty

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

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