Butyrylcholinesterase Fluorescent Activity Kit

Catalog Number EIABCHEF (192 tests)

Rev 1.0

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 Butyrylcholinesterase Fluorescent Activity Kit is a fluorescent activity assay designed to measure the activity of butyrylcholinesterase 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 BChE 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 BChE in serum, plasma (EDTA and heparin), CSF, or tissue/cell lysates. The assay was validated with human BChE, but is expected to measure BChE activity in samples from other species, including chicken, mouse, rat, dog, monkey, and pig.

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
Butyrylcholinesterase Standard; 200 mU/mL butyrylcholinesterase derived from human blood in a special 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
BChE Substrate; butyrylthiocholine iodide freeze dried with stabilizers	2 vials

Materials required but not supplied

- Distilled or deionized water
- Fluorescence microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 390 nm
- Calibrated adjustable precision pipettes and glass or 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.

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.

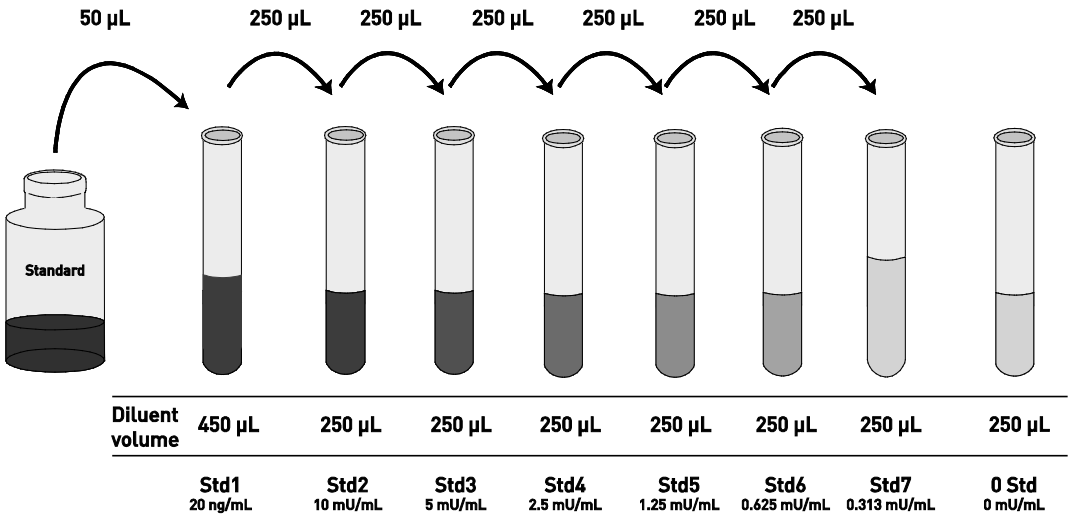
- Dilute **serum and plasma** samples ≥1:300 in 1X Assay Buffer.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: One unit of BChE hydrolyzes 1.0 µmol of butyrylcholine to choline and butyrate per minute at pH 8.0 and 37°C.

1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 50 µL Butyrylcholinesterase Standard to one tube containing 450 µL 1X Assay Buffer and label as 20 mU/mL BChE.
3. Add 250 µL 1X Assay Buffer to each of 7 tubes labeled as follows: 10, 5, 2.5, 1.25, 0.625, 0.313, and 0 mU/mL BChE.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **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.
   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 desiccated with the silica pack in the bag at 2°C to 8°C.
   Use within 2 weeks.

Reconstitute BChE Substrate

1. Add 700 µL of the Dry DMSO to the vial of BChE Substrate and vortex thoroughly.
   Note: Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.
2. The reconstituted BChE Substrate is a 10X concentrate. Store at room temperature. **Use within 2 weeks**.

Prepare reaction mix

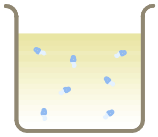
Prepare a reaction mix for detection of enzyme activity according to the table:

Reagent	½ plate	Full plate
10X BChE 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 (see page 2) into each well.
b. Tap the side of the plate to mix.
c. Incubate for 20 minutes at room temperature.

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 four parameter algorithm 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 0–20 mU/mL butyrylcholinesterase.

Standard BChE (mU/mL)	Mean FLU
20	59,868
10	32,329
5	16,480
2.5	8,706
1.25	4,988
0.625	3,136
0.313	2,093
0	1,054

Intra-assay precision

Samples of known butyrylcholinesterase concentration were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mU/mL)	5.70	2.97	1.17
%CV	4.7	7.3	7.5

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 16 times in duplicate by four operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mU/mL)	7.70	5.84	1.71
%CV	9.1	7.5	8.5

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

A variety of serum and plasma samples were tested with the assay. Values averaged 4,565 mU/mL.

Sample	Range (mU/mL)	Average (mU/mL)
Human serum/plasma (n=23)	—	6,268 ± 2,506
Rat serum/plasma (n=5)	293–365	—

Linearity of dilution

Linearity was determined by assaying 1:450 dilutions of high and low concentration plasma 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	3.33	3.24	97.2
60	40	5.88	5.60	95.3
40	60	8.42	7.61	90.4
20	80	10.97	9.37	85.4
			Mean Recovery	**92.1%**

Interferents

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

- 1% ethanol in the well decreased the activity by 12.6%, whereas 0.5% decreased activity by almost 10.3%.
- 5% DMSO in the well increased activity by 0.2%, while 1% increased activity by 6.5%.
- 10% methanol in the well increased activity by 0.6%
- 1% Triton™ X-100 in the well increased activity 4.0%.
- 0.1% hemoglobin in the well decreased activity 4.2%.

Sensitivity

The analytical sensitivity of the assay is 0.018 mU/mL butyrylcholinesterase. 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 acetylcholinesterase from the Acetylcholinesterase Fluorescent Activity Kit (Cat. No. EIAACHEF) resulting in a reading of < 0.2% of its expected activity.

Limited product warranty

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