Version 3a, Last updated 13 June 2025

ab241010 Butyrylcholinesterase Assay Kit

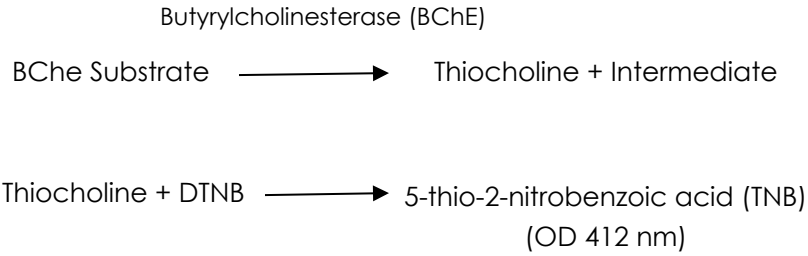
For the measurement of Butyrylcholinesterase activity in tissues or biological fluids.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Butyrylcholinesterase Assay Kit (ab241010) is based on the ability of Butyrylcholinesterase (BChE) to hydrolyze substrate and produce thiocholine. Thiocholine reacts with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) and generates a yellow chromophore that can be quantified at 412 nm. The assay is simple, sensitive and can detect as low as 0.2 U/ml in variety of samples.



Prepare Samples, Sample Background Controls and BChE Positive Control as described (total volume of 95 µL each). Prepare standard curve.

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Add DTNB (5 µL) to Samples, Sample Background Controls and BChE Positive Control and incubate for 10 minutes at room temperature protected from light.

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Add diluted BChE Substrate (100 µL) to Samples and BChE Positive Control. Add Assay Buffer 33 (100 µL) to Sample Background Controls.

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Shake plate. Read OD 412 in kinetic mode for 20-30 minutes at room temperature. TNB standard curve may be read in endpoint mode at 412 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 33	50 mL	-20°C	N/A
BChE Substrate	100 µL	-20°C	-20°C
BChE Positive Control	1 vial	-20°C	-20°C
DTNB	1 vial	-20°C	-20°C
TNB Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 33 was previously labelled as Assay Buffer XXXIII and BChE Assay Buffer. The composition has not changed.

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 412 nm
- 96 well plate with clear flat bottom
  Include if assay kit for tissue lysates/extracts
- Dounce homogenizer (if using tissue)
- Protease inhibitor cocktail for tissue samples

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer 33

Ready to use as supplied. Store at -20°C or 4°C. Warm to room temperature before use.

5.2 BChE Substrate

Aliquot and store at -20 °C, protect from light. Bring to room temperature before use.

5.3 BChE Positive Control

Reconstitute BChE Positive Control in 20 µL Assay Buffer 33. Store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within 2 months.

5.4 DTNB Solution

Dissolve DTNB in 625 µL Assay Buffer 33. Use within 2 months.

5.5 TNB Standard

Dissolve in 1 mL of Assay Buffer to generate 2.5 mM TNB Standard. The TNB standard solution is stable for at least 2 months at -20°C.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare 2.5 mM TNB standard as described in Section 5.5.
2. Add 0, 2, 4, 6, 8, 10, 12 µL of the 2.5 mM TNB Standard into 96-well plate in duplicate to generate 0, 5, 10, 15, 20, 25, 30 nmol/well standard. Bring the final volume to 200 µL with Assay Buffer 33.

Standard #	2.5 mM TNB Standard (µL)	Assay Buffer 33 (µL)	Final volume standard in well (µL)	TNB Standard in well (nmol/well)
1	0	200	200	0
2	2	198	200	5
3	4	196	200	10
4	6	194	200	15
5	8	192	200	20
6	10	190	200	25
7	12	188	200	30

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Serum, plasma or blood:

1. Prepare a 40-200-fold dilution of serum, plasma or blood in dH2O. Add 10-20 µL of diluted sample into desired well(s).
2. Prepare parallel sample well(s) as Sample Background Control.

ΔNote: Mix dilutions thoroughly by pipetting up and down after addition of biological fluids, since the density and viscosity cause sedimentation of sample to the bottom of the wells.

7.2 Tissue samples:

1. Homogenize tissue (10-30 mg) with 100 µL ice-cold Assay Buffer 33 containing protease inhibitor cocktail and keep on ice for 10 minutes. Centrifuge at 10,000 x *g* at 4 °C for 10 minutes to remove cell debris. Transfer the supernatant to a fresh tube. Add 5-20 µL sample per well.
2. Prepare parallel sample well(s) as Sample Background Control.

7.3 BChE Positive Control:

1. Prepare a 50-fold dilution of BChE Positive Control solution (i.e. dilute 1 µL of BChE Positive Control stock solution with 49 µL Assay Buffer 33). Add 8-12 µL of Diluted BChE Positive Control into well(s) assigned as BChE Positive Control.

ΔNote: Adjust the volume of sample(s), background control(s) and Positive Control to 95 µL/well Assay Buffer 33.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 DTNB Reaction:

1. Each well (samples, samples background controls, positive control) should contain 95 µL total volume (see Section 7).
2. Add 5 µL of DTNB solution to each well (final volume 100 µL).
3. Incubate plate for 10 minutes at room temperature to achieve temperature equilibrium and complete the reaction of sample proteins' sulfhydryl groups with DTNB. Avoid light.

8.2 BChE Substrate preparation:

1. Prepare a 120-fold dilution of BChE Substrate (i.e. Dilute 5 µL of BChE stock substrate with 595 µL of Assay Buffer 33), vortex briefly and keep on ice.
2. Add 100 µL of Diluted BChE substrate to each well containing the test samples and BChE Positive Control. Mix well
3. For Sample Background Control, add 100 µL of Assay Buffer 33 into assigned well(s).

ΔNote: The total volume in every well (i.e. standards, samples, background controls) should be 200 µL.

8.3 Measurement:

1. Measure absorbance immediately at 412 nm in kinetic mode for 20-30 minutes at room temperature.
2. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).
3. The TNB Standard Curve (Section 6) can be read in endpoint mode.

ΔNote: Shake the microplate carefully for 10 seconds to mix contents prior to start of read-out.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract 0 Standard reading from all readings. Plot the TNB Standard Curve.
3. Calculate the BChE activity of the test sample: $\Delta OD = OD_2 - OD_1$.
4. Apply the ΔOD to the TNB Standard Curve to get B nmol of TNB generated during the reaction time ($\Delta t = t_2 - t_1$).
5. Subtract the sample background control reading from its paired sample reading ($B_{test\ sample}/\Delta t - B_{sample\ background\ control}/\Delta t$)

$$Sample\ BChE\ activity\ (A) = \frac{Btest\ sample - Bsample\ control}{\Delta t * V} * D$$

Where:

A= BChE activity (nmol/minute/mL = mU/mL).

B = amount of TNB in the sample well calculated from standard curve (nmol).

V = sample volume added in the sample wells (mL).

D = sample dilution factor.

Unit definition: One unit of BChE activity is the amount of enzyme that generates 1.0 µmol of Thiocholine per minute at pH 7.4 at room temperature.

10. Typical Data

Data provided **for demonstration purposes only.**

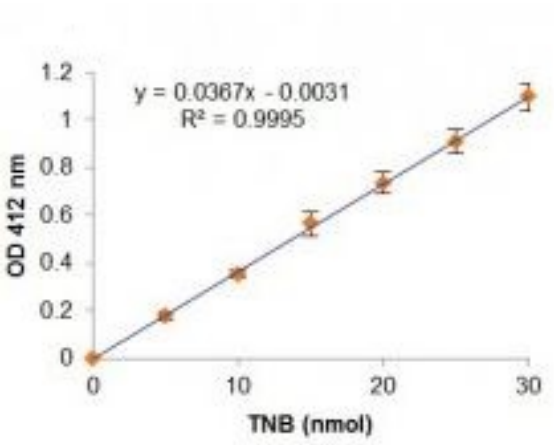

Figure 1.TNB standard curve.

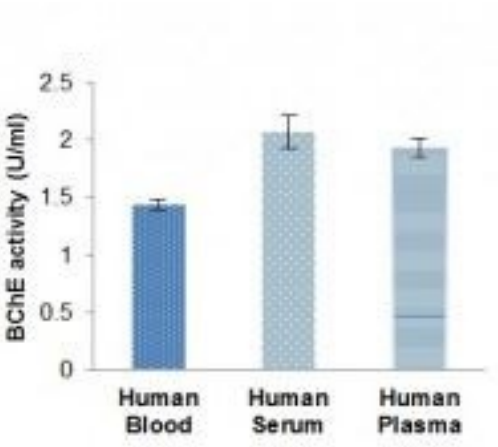

Figure 2. BChE activity in human blood (10 µL, 1:100 dilution), human serum (10 µL, 1:50 dilution) and human plasma (10 µL, 1:50 dilution).

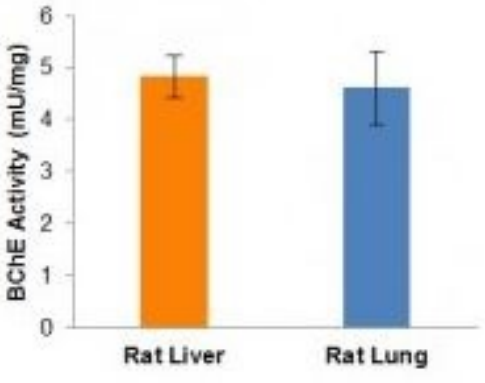

Figure 3. BChE activity in rat liver (30 µg protein) and rat lung (15 µg protein).

11. Notes

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)