abcam®
discover more

ab65345

Choline/Acetylcholine Assay kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Choline/Acetylcholine levels in various samples.

View kit datasheet: www.abcam.com/ab65345
(use www.abcam.cn/ab65345 for China, or www.abcam.co.jp/ab65345 for Japan)

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

Version 12b Last Updated 30 June 2025

Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 3

GENERAL INFORMATION

3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

ASSAY PREPARATION

9. REAGENT PREPARATION 8
10. STANDARD PREPARATION 9
11. SAMPLE PREPARATION 11

ASSAY PROCEDURE and DETECTION

12. ASSAY PROCEDURE and DETECTION 12

DATA ANALYSIS

13. CALCULATIONS 15
14. TYPICAL DATA 16

RESOURCES

15. QUICK ASSAY PROCEDURE 19
16. TROUBLESHOOTING 20
17. FAQs 23
18. INTERFERENCES 24
19. NOTES 25

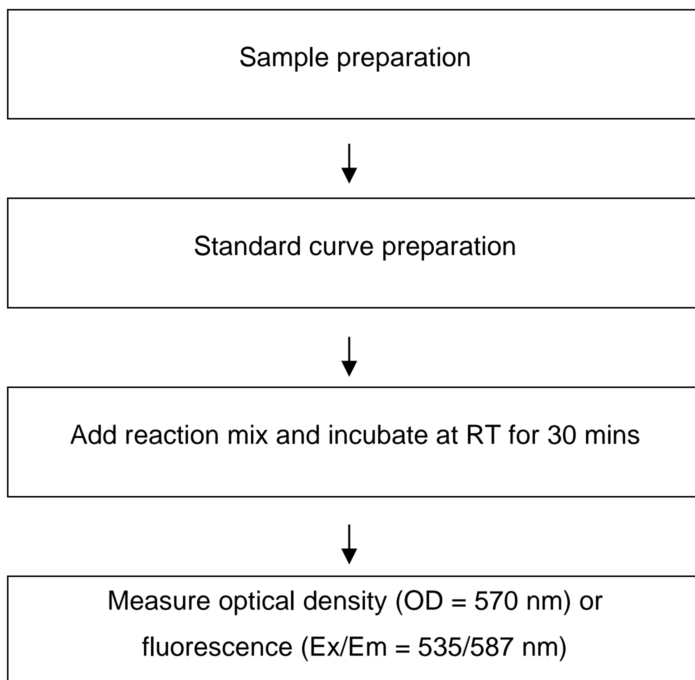
1. BACKGROUND

Choline/Acetylcholine Assay Kit (Colorimetric/Fluorometric) (ab65345) is a simple and sensitive assay for quantifying choline and acetylcholine by either a colorimetric or fluorometric method. In the assay free choline is oxidized via the intermediate betaine aldehyde to betaine, a methylglycine metabolite involved in osmolysis to protect cells under stress and which also functions as catabolic source of methyl groups for use in many biochemical pathways via transmethylation. The reaction generates products which react with the OxiRed™ Probe to generate color (λ= 570 nm), and fluorescence (Ex/Em 535/587 nm). Acetylcholine can be converted to choline by adding Acetylcholinesterase Enzyme to the reaction.

This kit can detect choline and acetylcholine (total choline – free choline) in various biological samples such as in blood, cells, culture media, fermentation media, etc. There is no need for pre-treatment or purification of samples. The kit can detect ~10 pmol-5 nmol of choline or acetylcholine.

Choline and acetylcholine play important roles in many biological processes. Choline is an essential nutrient, commonly grouped with the B complex vitamins, that plays key roles in many biological processes. Choline is a precursor for the synthesis of acetylcholine (AchE), a critical neurotransmitter in both the peripheral and central nervous systems.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 6	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Enzyme Mix IV	1 vial	-20°C	-20°C
Acetylcholinesterase Enzyme	1 vial	-20°C	-20°C
Choline Standard	1 vial	-20°C	-20°C

PLEASE NOTE: OxiRed™ Probe was previously labelled as OxiRed Probe and Choline Probe (in DMSO), and Assay Buffer 6 as Assay Buffer VI and Choline Assay Buffer. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

7. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

8. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 6:**

Ready to use as supplied. Warm to room temperature before use. Store at -20°C, protect from light and moisture.

9.2 **Choline Standard:**

Reconstitute standard in 100 µL Assay Buffer 6 to generate a 50 nmol/ µL Choline Standard solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

9.3 **OxiRed™ Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light and moisture. Once the OxiRed™ Probe is thawed, use with two months.

9.4 **Enzyme Mix IV:**

Dissolve in 220 µL Assay Buffer 6. Pipette up and down several times to ensure it is dissolved. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C.

9.5 **Acetylcholinesterase Enzyme:**

Dissolve in 220 µL Assay Buffer 6. Pipette up and down several times to ensure is dissolved. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C. Use within two months.

STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare a 0.5 nmol/µL Choline Standard by diluting 5 µL of 50 nmol/µL Choline Standard in 495 µL of Assay Buffer 6.

10.1.2 Using 0.5 nmol/µL Choline standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.5 nmol/µL Standard (µL)	Assay Buffer 6 (µL)	Final volume standard in well (µL)	End [Choline] in well
1	0	150	50	0 nmol/well
2	6	144	50	1 nmol/well
3	12	138	50	2 nmol/well
4	18	132	50	3 nmol/well
5	24	126	50	4 nmol/well
6	30	120	50	5 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

10.2 **For the flurometric assay:**

10.2.1 Prepare a 0.5 nmol/µL Choline Standard by diluting 5 µL of 50 nmol/µL Choline Standard in 495 µL of Assay Buffer 6.

10.2.2 Prepare 50 pmol/µL Choline Standard by diluting 10 µL of 0.5 nmol/µL Choline Standard into 90 µL Assay Buffer 6.

10.2.3 Using 50 pmol/µL Choline standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 50 pmol/µL Standard (µL)	Assay Buffer 6 (µL)	Final volume standard in well (µL)	End [Choline] in well
1	0	150	50	0 pmol/well
2	6	144	50	100 pmol/well
3	12	138	50	200 pmol/well
4	18	132	50	300 pmol/well
5	24	126	50	400 pmol/well
6	30	120	50	500 pmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL)

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

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. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend the cell pellet in 500 µL of Assay Buffer 6.

11.1.4 Homogenize by pipetting up and down a few times and leaving the cells on ice for 10 min.

11.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 500 - 700 µL of Assay Buffer 6.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice

11.3 Plasma

Typical level of choline in human plasma is about 9 – 13 µM. It is recommended to use the fluorometric approach with plasma samples to achieve higher sensitivity.

Plasma must be collected in heparinized tubes and stored in -80°C for no more than 1 month. Citrate can also be used for collection. Avoid EDTA as anticoagulant.

Typically, plasma samples don't need to be diluted. Use 2 – 20 µL of the plasma directly in the assay well.

11.4 Serum and Urine and other biological fluids:

11.4.1 Cells should be cultured in choline chloride free medium. Blood cells, culture media, fermentation media can be used in the assay without pretreatment or purification. Dilute in Assay Buffer 6 and use directly in the assay.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range. For human serum, use 10 – 25 µL of sample as human serum contains ~10µM choline. Free choline in serum is known to increase upon storage due to breakdown of lipids.*

ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

NOTE: This assay can detect choline and acetylcholine. If you want to detect free choline only, omit the Acetylcholinesterase Enzyme from the Reaction Mix as shown in step 12.3. With the addition of Acetylcholinesterase Enzyme, the assay detects total choline (free choline + acetylcholine (step 12.2)).

NOTE: If you want to measure both choline and acetyl choline, only 1 standard curve is required, as acetylcholine is converted to choline during the reaction.

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 6). (If both free choline and choline +AchE are to be measured, additional sample wells will be required).
- Background wells = 50 µL Assay Buffer 6.

12.2 **Choline + AchE Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (choline + AchE) (µL)	Fluorometric Reaction Mix (choline + AchE) (µL) *
Assay Buffer 6	44	45.6
OxiRed™ Probe*	2	0.4
Acetycholinesterase	2	2
Enzyme Mix IV	2	2

12.3 **Free Choline only Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (free choline) (µL)	Fluorometric Reaction Mix (free choline) (µL) *
Assay Buffer 6	46	47.6
OxiRed™ Probe*	2	0.4
Enzyme Mix IV	2	2

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

****NOTE:** The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µL of the OxiRed™ Probe per reaction to decrease the background reading and increase detection sensitivity.*

12.4 Add 50 µL of standard and samples to the wells.

12.5 Add 50 µL of Reaction mix to all standard, sample and background wells.

12.6 Mix and incubate at room temperature for 30 minutes protected from light.

12.7 Measure on a microplate reader.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay measure Ex/Em = 535/587 nm.

9. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Choline.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Cho = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.6 Concentration of choline in the test samples is calculated as:

$$Choline\ Concentration = \frac{Cho}{Sv} * D$$

Where:

Cho = amount of choline in sample well from the standard curve.

Sv = volume of sample used in the reaction (mL).

D = Sample dilution factor.

To calculate the concentration of Acetylcholine in the sample, use the following formula:

$$Acetylcholine = Total\ choline - Free\ choline$$

10. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

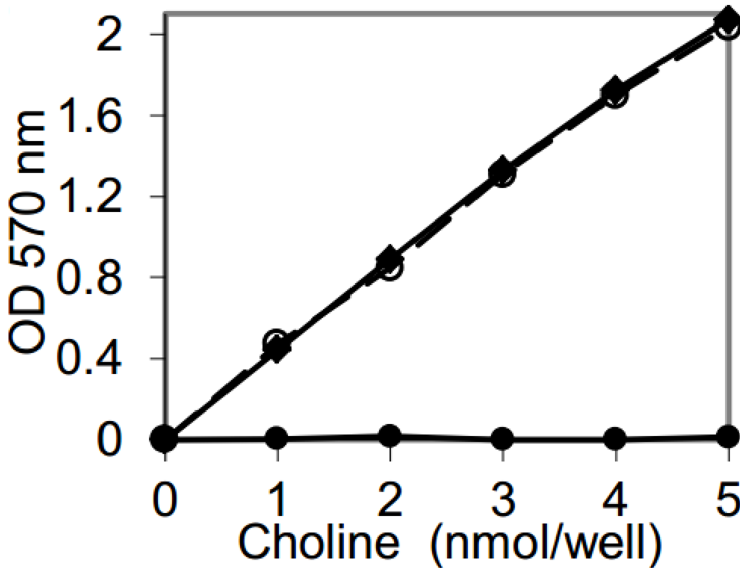


Figure 1: Typical choline standard calibration curve using colorimetric reading. (Legend: diamonds generated using choline as the substrate; open and closed circles were generated using acetylcholine as substrate in the presence and absence of Acetylcholinesterase Enzyme).

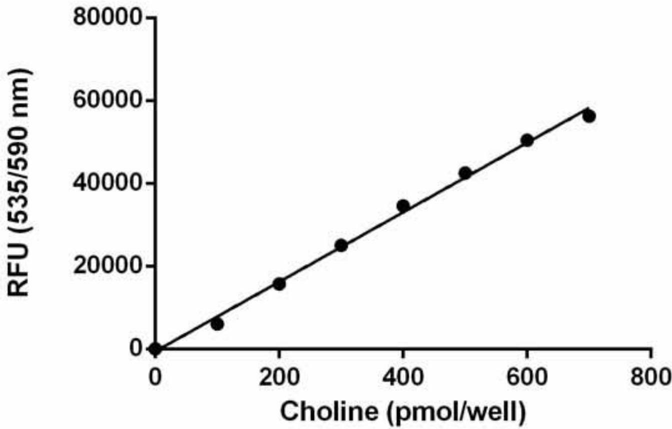


Figure 2: Typical choline standard calibration curve using fluorometric reading.

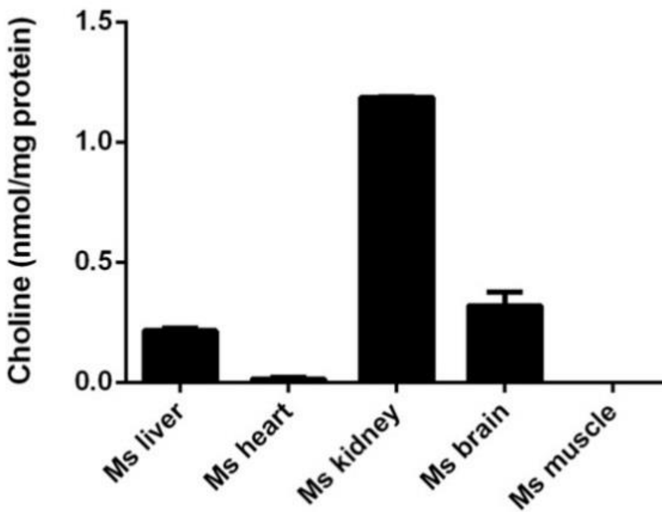


Figure 3: Choline measured fluorometrically in mouse tissue lysates showing quantity (nmol) per mg protein of tested sample.

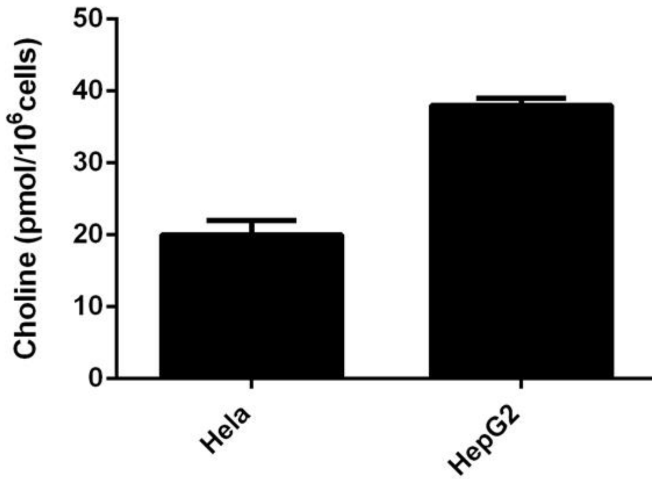


Figure 4: Choline measured fluorometrically in cell lysates showing quantity (pmol) per 1 mln of tested cells.

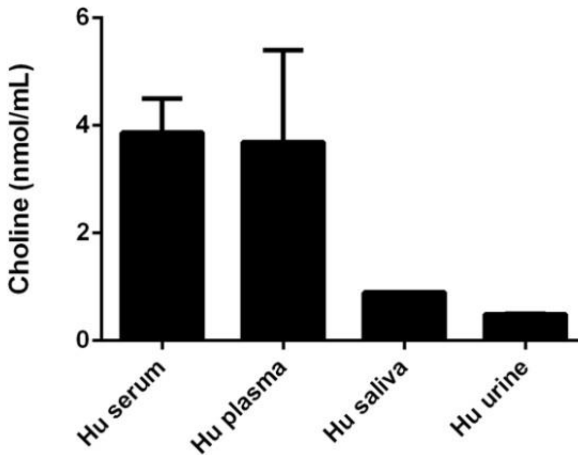


Figure 5: Choline measured fluorometrically in biological fluids showing quantity (nmol) per mL of tested sample.

11. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, OxiRed™ Probe, Acetylcholinesterase Enzyme and Enzyme Mix IV/enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings) – additional sample wells required if measuring Free Choline and Choline + AchE.
- Prepare appropriate Reaction Mix (based on Number samples + standards + 1) as follows:
  - Choline + AchE Reaction Mix:

Component	Colorimetric Reaction Mix (choline + AchE) (µL)	Fluorometric Reaction Mix (choline + AchE) (µL)
Assay Buffer 6	44	45.6
OxiRed™ Probe	2	0.4
Acetycholinesterase Enzyme	2	2
Enzyme Mix IV	2	2

  - Free Choline only Reaction Mix:

Component	Colorimetric Reaction Mix (free choline) (µL)	Fluorometric Reaction Mix (free choline) (µL)
Assay Buffer 6	46	47.6
OxiRed™ Probe	2	0.4
Enzyme Mix IV	2	2

- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Add 50 µL of appropriate Reaction Mix to wells.
- Incubate plate at RT for 30 minutes protected from light.
- Measure plate at OD 570nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

- **TROUBLESHOOTING**

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of inappropriate plate for reader	Colorimetry: Clear plates Fluorescence: Black plates (clear bottom)
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range

12. FAQs

How much cell lysate proteins should be used for this assay?

Typically we suggest using 100-200 µg for each assay. However, amount may vary depending on the Choline/Acetyl choline content in the tissue and cells samples.

Can milk powder be used as a sample for this kit?

Milk powder would have to dissolved homogeneously in the Assay Buffer 6 (it is critical to ensure there are no particles or clumps) and then different dilutions can be prepared in the assay buffer and used for the assay.

Why is there under-estimation of choline content from standards spiked with serum samples?

There are serum proteins/enzymes that bind to choline/acetyl choline and hence it is possible that the recovery based on the calculations from this kit is lower than the expected value. Deproteinizing the serum sample could help.

The total choline ODs are the same as the free choline ODs, suggesting the ACh esterase is inactive. Why?

There are a number of explanations for this: (1) Exposure to many drugs can lead to serum inhibition of the acetylcholine esterase enzyme, especially in human samples. (2) There are extremely low levels of free acetyl choline in the samples (this is not unusual) and hence after addition of the esterase the total choline level is equal to the free choline. (3) It would help to measure the background for the free and total choline so that after background subtraction the values can be compared.

Can protein content be used as an internal control for this assay?

Yes, a detergent-compatible BCA assay can be used for protein quantitation to normalize sample amount.

13. INTERFERENCES

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Avoid EDTA when preparing plasma or serum samples.

14. NOTES

abcam®
discover more

Technical Support
Copyright © 2025 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.
For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)