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ab219944
Choline Detection Kit

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

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

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1. Overview

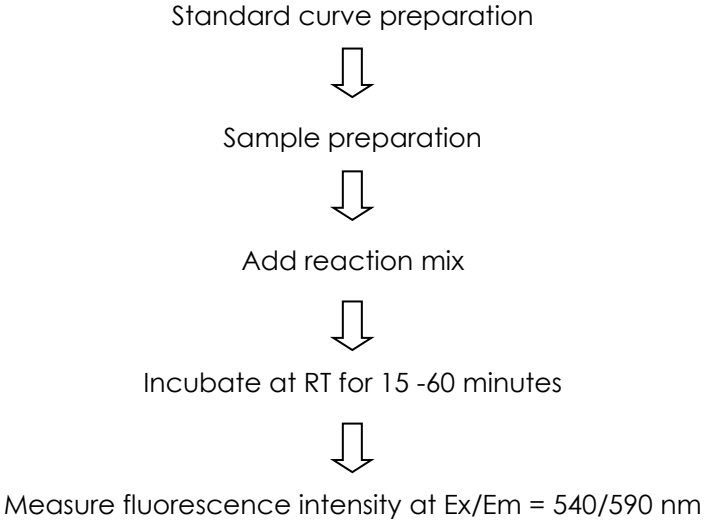
Choline Detection Kit (ab219944) provides a sensitive fluorescence-based assay for quantifying choline in cell and tissue lysates and biological fluids.

The amount of choline is proportional to the concentration of hydrogen peroxide formed in the choline oxidase-mediated enzyme coupling reaction cycle, which in turn is detected with our proprietary Red Indicator. The increase in fluorescence can be easily detected at an optimal Ex/Em = 540/590 nm. The signal can also be read by absorbance at OD: 576 ± 5 nm, although the sensitivity of the assay is reduced 10-fold.

The assay detects as little as 4 picomole choline in 100 µL assay volume (40 nM), making it one of the most sensitive assays available in the market.

Choline is an essential nutrient. Choline and its metabolites play an important role in the structural integrity and signaling of cell membranes and cholinergic neurotransmission (choline synthesis). It is a major source of methyl group via its metabolite, trimethylglycine that participates in the S-adenosylmethionine synthesis pathways. Choline deficiency may cause liver disease, atherosclerosis and possibly neurological disorders. Despite its importance in the central nervous system as a precursor for acetylcholine and membrane phosphatidylcholine, the role of choline in mental illness has been little studied.

2. Protocol 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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

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

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer	25 mL	-20°C	-20°C
AbRed Indicator	1 vial	-20°C	-20°C
Choline Probe (lyophilized)	2 vials	-20°C	-20°C
Choline Standard	2.8 mg	-20°C	-20°C
DMSO	100 µL	-20°C	-20°C

7. 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 fluorescence at Ex/Em = 540/590 nm
- Double distilled water (ddH_2O)
- Choline chloride free culture medium
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well solid black plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- (Optional)Mammalian Cell Lysis Buffer 5X (ab179835)

8. 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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 Choline Probe:

Immediately prior to use, add 5 mL of Assay Buffer to one vial of Choline Probe. Mix well by pipetting up and down.

Δ Note: reconstituted Choline Probe is enough for 1 x 96 well plate. Reconstituted probe cannot be stored for future use.

Store un-reconstituted probe at -20°C.

9.3 Choline Standard:

Reconstitute the Choline Standard solution in 400 µL of ddH_2O to generate a 50 mM Choline Standard stock solution. Mix well by pipetting up and down. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

9.4 DMSO:

Ready to use as supplied. Warm by placing in a 37°C bath for 1-5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Store at -20°C protected from light. Avoid repeated freeze-thaw cycles.

9.5 Red Indicator:

Dissolve AbRed Indicator in 40 µL DMSO and mix thoroughly by pipetting up and down. Label this component **250X AbRed Stock Solution**. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze-thaw cycles. Use within two months.

10. Standard Preparation

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

10.1 Prepare a 1 mM Choline Standard solution by adding 10 µL of 50 mM choline standard stock solution (Step 9.3) to 490 µL Assay Buffer. Mix well.

10.2 Prepare a 30 µM Choline Standard solution by adding 30 µL of 1 mM choline standard solution (Step 10.1) to 970 µL Assay Buffer. Mix well.

10.3 Using the 30 µM Choline Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard#	Sample to dilute	Volume standard in well (µL)	Assay Buffer (µL)	End conc Choline in well
1	30	300	0	30 µM
2	Std #1	100	200	10 µM
3	Std #2	100	200	3 µM
4	Std #3	100	200	1 µM
5	Std #4	100	200	0.3 µM
6	Std #5	100	200	0.1 µM
7	Std #6	100	200	0.03 µM
8 (blank)	0	0	300	0 µM

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

Δ Note: Diluted choline Standard solution is unstable, and should be used within 4 hours.

11. 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 snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell lysates:

Δ Note: For ease of use, mammalian adherent or suspension cells lysates can be easily prepared using Mammalian Cell Lysis Buffer 5X (ab179835). Follow product protocol and proceed to Section 12.

Δ Note: Cells should be cultured in choline chloride free medium to minimize background signal.

11.1.1 Harvest the number of cells necessary for each assay (initial recommendation: 5 x 10^5 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend or scrape cells in 200 µL of cold Assay Buffer.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Centrifuge 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep on ice.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 20 mg).
11.2.2 Wash tissue with cold PBS.
11.2.3 Homogenize tissue in 400 µL Assay Buffer using a Dounce homogenizer.
11.2.4 Centrifuge homogenate at 2,500 rpm for 5 – 10 minutes at 4°C.
11.2.5 Transfer supernatant to a new tube.
11.2.6 Keep sample on ice.

11.3 Plasma:

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

11.4 Serum and Urine (and other biological fluids):

Samples can be used directly or diluted in Assay Buffer for testing.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- The protocol describe in this section is for 1 x 96-well plate. To perform the assay in a 384-wp, scale down volumes by half.

12.1 Reaction wells set up:

- Blank control = 50 µL Assay Buffer.
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2 Run Choline Assay:

12.2.1 Immediately prior using, add 20 µL of 250X Red Dye stock solution (Step 9.5) to the Choline Probe Solution (Step 9.2) to make the Choline Reaction mixture.

Δ **Note**: The Choline Reaction should be used promptly and kept protected from light. The assay background increases with longer storage time.

12.2.2 Add 50 µL of Choline Reaction Mix into each well (total volume = 100 µL/well).

12.2.3 Incubate reaction mixture at room temperature for 10-30 minutes, protected from light.

12.3 Measurement:

12.3.1 Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm).

13. Calculations

– 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.

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean fluorescence value of the blank (Standard #8) from all standard and sample readings. This is the corrected fluorescence.

13.3 Plot the corrected fluorescence 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 Apply the corrected sample RFU reading to the standard curve to get Choline concentration (µM) (C) in the sample wells.

13.6 Optionally, the amount of Choline (pmol) in the test samples is calculated as:

$$Amount\ of\ Choline = C * V * D$$

Where:

C = concentration of Choline in the sample well calculated from standard curve (µM).

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

D = sample dilution factor if sample is diluted to fit within the standard curve range.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

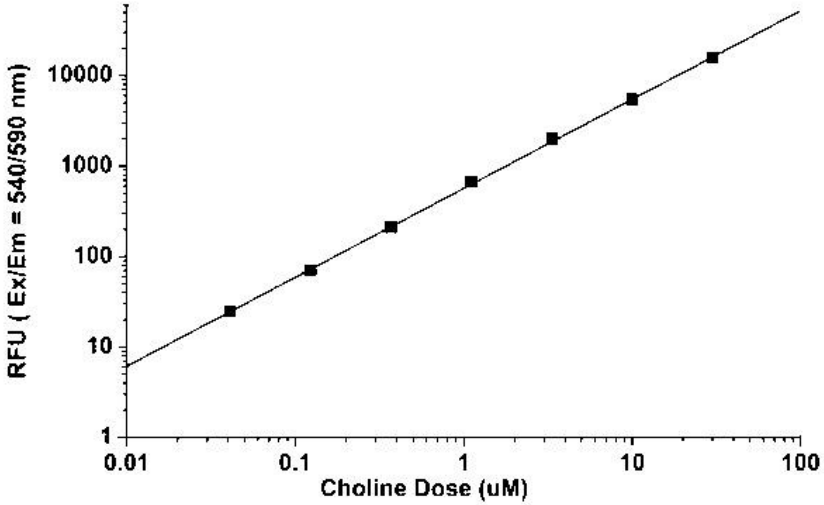


Figure 1. Typical Choline standard calibration curve. Choline dose response was measured on a 96 well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 40nM (4 picomole/well) of choline can be detected with 30 minutes incubation time (n=3).

15. Quick Assay Procedure

Δ 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 reagents and aliquot; get equipment ready.
- Prepare Choline standard dilution [30-0.03 µM].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL) and samples (50 µL).
- Prepare Choline Reaction Mix by adding 20 µL of 250X Red Dye stock solution to the Choline Probe Solution.
- Add 50 µL of Reaction Mixture into each well.
- Incubate plate at RT for 10-30 minutes.
- Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm).

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	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	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) 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 described in the 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 it is within the linear range

17. Notes

Technical Support

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