Elite™ Choline Quantitation Kit (Red Fluorescence)

CATALOG NUMBER: CA-C007, 200 assays

Description

Choline, a water-soluble substance, is used by the body to synthesize acetylcholine (a neurotransmitter), lecithin (phosphatidylcholine), and platelet-activating factor (a blood clotting agent). It prevents fat deposits in the liver and facilitates the movement of fats into the cells. Choline can be obtained from dietary sources as a supplement and is also synthesized by the body.

The Elite™ Choline Quantitation Kit provides one of the most sensitive methods for quantifying choline. The kit uses Choline™ Red to quantify the concentration of choline, which is related to the production of hydrogen peroxide in the choline oxidase-mediated enzyme coupling reactions. The amount of choline is proportional to the concentration of hydrogen peroxide formed in the enzyme coupling reaction cycle. In the presence of peroxidase, the fluorescence intensity of Choline™ Red is proportional to the formation of hydrogen peroxide that is converted to the concentration of choline. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments. It detects as little as 0.01 nmole choline in 100 µl assay volume (0.1 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read with a fluorescence microplate reader at Ex/Em = ~540/590 nm. Alternatively, the assay can also be read at ~576±5 nm with an absorbance microplate reader.

Kit Components

- **Component A:** Choline™ Red — 1 vial
- **Component B:** Choline Probe — 2 bottles (lyophilized powder)
- **Component C:** Choline Standard — 1 vial
- **Component D:** Assay Buffer — 1 bottle (25 ml)
- **Component E:** DMSO — 1 vial (100 µl)

Storage

Keep **Component A** in freezer (-20 °C) and avoid exposure to light, **Component B&C** at -20 °C, and **Component D&E** at 4 °C.

Materials Required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader

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Assay Protocol (for 96-well microplate)

Thaw all the kit components at room temperature before starting the experiment.

1. **Prepare stock solutions:**

   1.1 Choline™ Red stock solution (250X): Add 40 µl of DMSO (**Component E**) into the vial of Choline™ Red (**Component A**) to make a 250X stock solution.

   Note: *The unused Choline™ Red stock solution should be divided into single use aliquots. Store at -20 °C and avoid exposure to light.*

   1.2 Choline stock solution: Add 400 µl of ddH_2O into the vial of Choline Standard (**Component C**) to make 50 mM choline stock solution.

   Note: *The unused choline stock solution should be divided into single use aliquots and stored at -20 °C.*

2. **Prepare choline assay mixture:**

   2.1 Add 5 ml of Assay Buffer (**Component D**) to the bottle of Choline Probe (**Component B**) and mix well.

   2.2 Add 20 µl of Choline™ Red stock solution (250X, from Step 1.1) into the Choline Probe bottle (from Step 2.1) to make the choline assay mixture.

   Note: *The Assay mixture should be used promptly and kept from light. The assay background would increase with longer storage time.*

3. **Prepare serial dilutions of choline standard (0 to 30 µM):**

   3.1 Add 20 µl of 50 mM choline standard stock solution (from Step 1.2) to 980 µl Assay Buffer (**Component D**) to generate 1000 µM standard solution.

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

   3.2 Take 30 µl of 1000 µM standard (from Step 3.1) to 970 µl Assay Buffer (Component D) to generate 30 µM Choline Standard Solution, then perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, and 0 µM choline standard.

   3.3 Add the choline standards (CS) and the choline containing test samples (TS) into a 96-well solid black microplate as described in Tables 1 and 2.

   Note: *Treat the cells or tissue samples as desired.*

Table 1. Layout of choline standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	…							
CS1	CS1										
CS2	CS2										
CS3	CS3										
CS4	CS4										
CS5	CS5										
CS6	CS6										
CS7	CS7										

Note: CS=Choline Standards; BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

Choline Standards	Blank Control	Test Sample
Serial Dilutions: 50 µl	Assay Buffer: 50 µl	50 µl

Note: Add the serially diluted Choline standards from 0.03 to 30 µM into wells from CS1 to CS7 in duplicate.

4. **Run the choline assay:**

   4.1. Add 50 µl of choline assay mixture (from Step 2.2) to each well of the choline standard, blank control, and test samples (see Step 3.3) to make the total choline assay volume of 100 µl/well.

   Note: *For a 384-well plate, add 25 µl sample and 25 µl of choline assay mixture per well.*

   4.2. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

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

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the choline reactions. A choline standard curve is shown in Figure 1. The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

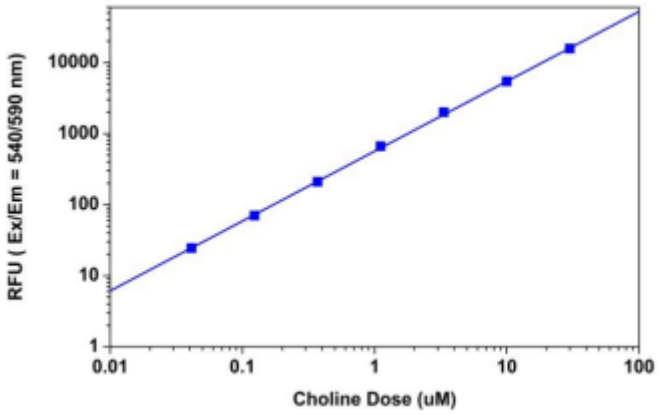

Figure 1. Choline dose response was obtained with Elite™ Choline Quantitation Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 100 nM (10 pmole/well) of choline can be detected with 30-minute incubation time (n=3).

References

1. Al-Saeedi F, Smith T, Welch A. (2007) [Methyl-3H]-choline incorporation into MCF-7 cells: correlation with proliferation, choline kinase and phospholipase D assay. Anticancer Res, 27, 901.
2. Park HD, Park KU, Kim KW, Song J, Chang HE, Heo SR, Lee HJ, Kim JQ. (2007) Real-time multiplex PCR assay for genotyping of three apolipoprotein E alleles and two choline acetyltransferase alleles with three hybridization probes. Clin Chem Lab Med, 45, 346.
3. Adamczyk M, Brashear RJ, Mattingly PG. (2006) Rapid high-throughput detection of peroxide with an acridinium-9-carboxamide: a homogeneous chemiluminescent assay for plasma choline. Bioorg Med Chem Lett, 16, 2407.
4. Shiba K, Ogawa K, Kinuya S, Yajima K, Mori H. (2006) A simple and rapid radiochemical choline acetyltransferase (ChAT) assay screening test. J Neurosci Methods, 157, 98.
5. Panfili G, Manzi P, Compagnone D, Scarciglia L, Palleschi G. (2000) Rapid assay