Amplite™ Choline Quantitation Kit
*Red Fluorescence*

### Ordering Information:
- **Product Number:** 40007 (200 assays)
- **Storage Conditions:** Keep in freezer
  Avoid light
- **Instrument Platform:** Fluorescence microplate readers

### Introduction
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 phosphatidylycholine, the role of choline in mental illness has been little studied.

Our Amplite™ Choline Quantitation Kit provides one of the most sensitive methods for quantifying choline. The kit uses Amplite™ 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. The kit is an optimized “mix and read” assay that is compatible with HTS liquid handling instruments. It detects as little as 10 picomole choline in 100 µL assay volume (100 nM) as shown in Figure 1. 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 Key Features
- **Broad Application:** Can be used for quantifying choline in solutions and in cell extracts.
- **Sensitive:** Detect as low as 10 picomole of choline in solution.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time.

### Kit Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A: Amplite™ Red</td>
<td>1vial</td>
</tr>
<tr>
<td>Component B: Choline Probe</td>
<td>2 bottles (lyophilized powder)</td>
</tr>
<tr>
<td>Component C: Choline Standard</td>
<td>1vial (2.8 mg)</td>
</tr>
<tr>
<td>Component D: Assay Buffer</td>
<td>1 bottle (25 mL)</td>
</tr>
<tr>
<td>Component E: DMSO</td>
<td>1vial (100µL)</td>
</tr>
</tbody>
</table>

### Assay Protocol for One 96-well Plate

**Brief Summary**

Prepare choline assay mixture (50 µL) → Add choline standards or choline test samples (50 µL) → Incubate at RT for 15-60 min → Read fluorescence intensity at Ex/Em = 540/590 nm

*Note: Thaw all the kit components at room temperature before starting the experiment.*
1. Prepare stock solutions:

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

*Note: The unused Amplite™ 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 ddH₂O 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 Amplite™ 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 choline 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, and 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 choline standards and choline containing test samples 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 Standard | Blank Control | Test Sample |
| Serial dilutions* (50 μL) | Assay buffer: 50 μL | 50 μL |

*Note: Add the serially dilutions of choline standards from 0.03 to30 μM into wells from CS1 to CS7 in duplicate.

4. Run 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.

![Choline Standard Curve](image)

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

References