Acetylcholine Fluorometric/Colorimetric Assay Kit

Catalog No. KM0001

Detection and Quantification of Acetylcholine Concentrations in Biological Samples.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.
INTRODUCTION

Acetylcholine, ACh, is a neurotransmitter that is present in the central nervous system as well as the peripheral nervous system. Acetylcholine is involved in the cholinergic system which causes anti-excitatory actions in the central nervous system. In the peripheral nervous system, acetylcholine is found to regulate and control muscle movement. The acetylcholine receptors can be regulated by drugs that may act as either agonists or antagonists.

In this assay, acetylcholinesterase catalyzes the reaction of acetylcholine to choline. Choline then gets oxidized to H$_2$O$_2$ by choline oxidase. In the presence of horseradish peroxidase (HRP), the H$_2$O$_2$ reacts with a specialized fluorescent probe in a 1:1 stoichiometry to generate the red-fluorescent oxidation product, resorufin. Resorufin has fluorescence excitation and emission maxima of approximately 571 nm and 585 nm and absorbance maxima at 570 nm.

FEATURES

**Number of Assays:** 100 Assays

**Samples:** serum, plasma, urine, milk, saliva, cell lysates, tissue lysates, etc.

Note: This assay kit is validated with serum.

**Fluorometric Dynamic Range:** 3 to 200 uM Acetylcholine.

Fluorescence at Ex/Em = 540/600 nm

**Colorimetric Dynamic Range:** The colorimetric range is typically 10X less sensitive than the fluorometric range.

Absorbance at 570 nm

STORAGE & HANDLING

Upon receipt, take out the Reaction Buffer and Acetylcholine Standard; store at 4°C. Take out the microplate and leave at room temperature. Store all other components in the original box at -20°C, protected from light. The kit should be stable for at least six months.

**Note:** Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
**KIT CONTENTS & STOCK PREPARATION**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Storage</th>
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<tbody>
<tr>
<td>96-Well Microplate</td>
<td>1 plate</td>
<td>RT</td>
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<tr>
<td>Reaction Buffer</td>
<td>25 ml</td>
<td>4°C</td>
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<td>Acetylcholine Standard</td>
<td>1 ml of 2 mM</td>
<td>4°C</td>
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<td>Reaction Mix (Green Cap)</td>
<td>1 vial, lyophilized</td>
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<tr>
<td>Dye Reagent (Red Cap)</td>
<td>250 ul</td>
<td>-20°C</td>
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Note: If not running entire plate at once, aliquot and store reagents under conditions according to this table. Use within 2 months.

**MATERIALS NOT PROVIDED**

Pipetting devices, tubes, and microplate reader.

**ASSAY RESTRICTIONS**

- This kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in performance of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
REAGENT & SAMPLE PREPARATIONS

Please read the manual in its entirety before proceeding with the assay.

**Note:** Warm all reagents to room temperature before use and protect from light. Calculate and prepare the amount needed if a full plate is not used.

1. **Preparation of Standard**
   Acetylcholine Standard is provided at a 2 mM stock concentration.

   **Fluorometric Method:** To prepare the standard, dilute the appropriate amount of Acetylcholine stock solution in Reaction Buffer, starting from a high point of 200 μM down to a low point of 3 μM to create 7 concentrations for the standard. The last row consists of only Reaction Buffer to serve as a negative control. The standards are run in duplicate. Use a 96-well black, flat bottom plate.

   **Colorimetric Method:** The dynamic range for colorimetric method is typically 10 folds less sensitive than fluorometric methods. Follow the same protocol as the fluorometric method. It is advised to run a few standard concentration points to determine the range. To prepare the standard, dilute the appropriate amount of Acetylcholine stock solution in Reaction Buffer to create 7 concentrations for the standard. The last row consists of only Reaction Buffer to serve as a negative control. The standards are run in duplicate. Use a 96-well clear, flat bottom plate.

2. **Preparation of Samples**
   Dilute the samples in Reaction Buffer. A variable dilution will be required depending on the total amount of Acetylcholine present in your sample. In the first trial, the samples should be serially diluted to determine the optimal amount of sample for the assay. For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

   **Note:** Extremely high levels of Acetylcholine can produce lower fluorescence than moderately high levels due to excess H₂O₂ oxidizing the reaction product, resorufin, to non-fluorescent resazurin.

3. **Preparation of Reaction Mix**
   Reconstitute Reaction Mix with 5.76 ml of Reaction Buffer. If a full plate is used, put the reconstituted Reaction Mix on ice while in use. If not all of it is used, aliquot and store at -200°C. Avoid multiple freeze and thaw cycles.

4. **Preparation of Working Solution**
   Add 240 ul of Dye Reagent in the 5.76 ml reconstituted Reaction Mix to make the Working Solution. Mix well by inversion. Put on ice and protect from light since this solution is temperature and light sensitive. This solution cannot be stored, so make the exact amount needed for the experiments.
ASSAY PROTOCOL

1. Add 50 ul of each sample/standard to each well in a 96-well flat bottom microplate. The standards are run in duplicate. It is advised to run the samples in duplicate or triplicate.

Sample Plate Layout (you do not have to follow this)

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Note: BL=Blank Control, ST=Acetylcholine Standards, EX=Experimental Test Samples

2. Add 50 ul of the Working Solution to each well of the microplate containing the standards, controls, and samples.

3. Incubate at room temperature for 10 minutes, protected from light.

4. Read the plate using a microplate reader at Ex/Em: 540/600 nm for the Fluorometric Method or at 570 nm for the Colorimetric Method.

Because the assay is continuous (not terminated), the plate can be measured at multiple time points to follow the kinetics of the reaction.
STANDARD CURVE

Correct for background fluorescence or absorbance. For each point, subtract the value derived from the negative control. Plot the Acetylcholine concentrations vs. the read values to create the standard curve.

Acetylcholine Fluorometric/Colorimetric Assay Kit allows for the detection and quantification of endogenous Acetylcholine concentrations within the range of 3.125 uM - 200 uM in cell lysates, sera, and plasma.

CALCULATION

Determine the slope of the standard curve and calculate the Acetylcholine concentration of your samples.

\[
[\text{Acetylcholine}] = \left(\frac{\text{RFUsample} - \text{RFUblank}}{\text{Slope}}\right) \times \text{Dilution Factor}
\]

Note: Use OD values for colorimetric method

Conversions: 200 uM Acetylcholine = 2.92 mg/dl = 0.0029 % = 29.2 ppm
TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

NOTES