Butyrylcholinesterase Activity Assay Kit

ARG83560 Butyrylcholinesterase Activity Assay Kit can be used to measure Butyrylcholinesterase Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83560

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83560 Butyrylcholinesterase Activity Assay Kit provides a simple and sensitive method for monitoring Butyryl Cholinesterase activity in various samples. The enzyme catalysed reaction products p-nitrophenol can be measured at a colorimetric readout at 412 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4 °C
Positive Control	1 vial (lyophilized)	-20 °C
Reaction Buffer	20 ml	4 °C
Substrate	1 vial (lyophilized)	4 °C
Assay Buffer	4 x 30 ml	4 °C
Dye Reagent	1 vial (lyophilized)	4 °C
Plate sealer	3 adhesive strips	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 412 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Ethanol

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and Bacteria-Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per 5 × 10^6 cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** Reconstitute the **Substrate** with **1 ml** of distilled water. Allow the **Substrate** keep on bench for few minutes. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use.
  The Reconstitute **Substrate** is stable for 4 weeks at 4°C.
- **Dye Reagent:** Reconstitute the **Dye Reagent** with **1 ml** of ethanol. Allow the **Dye Reagent** keep on bench for few minutes. Make sure the **Dye Reagent** is dissolved completely and mixed thoroughly before use
  The Reconstitute **Dye Reagent** is stable for 4 weeks at 4°C.

- **Positive Control:** Reconstitute the **Positive Control** with **1 ml** of Assay Buffer. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use.
  The Reconstitute Positive Control is stable for 4 weeks at-80°C.

- **Standard:** Reconstitute the **Standard** with 1 ml of distilled water. Allow the **Standard** keep on bench for few minutes. Make sure the **Standard** is dissolved completely and mixed thoroughly before use.
  The Reconstitute **Standard** is stable for 4 weeks at-20°C.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add 170 µl **Reaction Buffer** into all wells.
2. Add 10 µl **Substrate** into Sample, Control and Positive Control wells.
3. Add 10 µl **Sample** into Sample wells.
4. Add 10 µl **Distilled water** into Control and standard wells.
5. Add 20 µl **Distilled water** into blank wells.
6. Add 10 µl **Standard** into Standard wells.
7. Add 10 µl **Positive Control** into Positive Control wells.
8. Add 10 µl **Dye Reagent** into each wells.
9. Mix well Incubate for 10 min at RT. Read the OD at 412 nm.

Summary of Butyrylcholinesterase Activity Assay Kit Procedure

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	170 µl	170 µl	170 µl	170 µl	170 µl
Substrate	10 µl	10 µl	-	-	10 µl
Sample	10 µl	-	-	-	-
Distilled water	-	10 µl	10 µl	20 µl	-
Standard	-	-	10 µl	-	-
Positive Control	-	-	-	-	10 µl
Dye Reagent	10 µl	10 µl	10 µl	10 µl	10 µl
Mix well Incubate for 2 min at RT Read the OD at 412 nm					

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition: One unit of Butyryl Cholinesterase activity is defined as the enzyme hydrolyze 1 µmol of butyrylthiocholine iodide per minute at pH 7.4 and 25°C.

$C_{Standard}$: the concentration of standard, 5 µmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 104;

$V_{Standard}$: the volume of the standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

b.) Calculation:

Formula:

a). According to the protein concentration

Butyrylcholinesterase (U/mg) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

$= 2.5 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

b). According to the weight

Butyrylcholinesterase (U/g) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W / V_{Assay}) \times T]$

$= 2.5 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the quantity of cell or bacteria

Butyrylcholinesterase ($U/10^4$) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times N / V_{Assay}) \times T]$

$= 2.5 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times N]$

4. According to the volume

Butyrylcholinesterase (U/ml) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T$

$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$