BUTYRYL CHOLINESTERASE

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1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Butyryl cholinesterase

1.2 **Systematic Name**: Acylcholine acylhydrolase

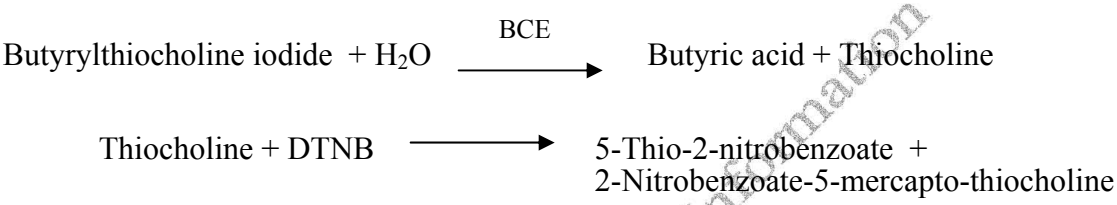
1.3 **E.C. Number**: E.C.3.1.1.8

1.4 **Source**: Equine serum

1.5 **Suitable for Biozyme codes**: BCE1, BCE3, BCE3S

2.0 ASSAY PRINCIPLE

The method of assay is based on that of Weber[1] in which the thiocholine formed by the enzymic hydrolysis of butyryl thiocholine is measured using 5',5'-Dithiobis(2-nitrobenzoic acid (DTNB) as indicator.



The rate of appearance of the yellow derivative (5-Thio-2-nitrobenzoate) is measured spectrophotometrically at 405nm.

3.0 UNIT DEFINITION

That amount of enzyme causing the hydrolysis of one micromole of butyrylthiocholine iodide per minute at 25°C and pH 7.4.

4.0 EQUIPMENT REQUIRED

Double beam UV/vis recording spectrophotometer, with temperature control set at 25°C (± 0.1°C).
Water bath at 25°C (± 0.5°C), thermometer.
Silica and plastic cuvettes, plastic test tubes, automatic pipettes and tips.

5.0 REAGENTS REQUIRED

When using the following reagents please refer to the manufacturer's instructions for safe handling and disposal.

Reagent details

DTNB
Supplier: Sigma
Product No.: D8130
F.W.: 396.4

Butyrylthiocholine iodide
Supplier: Sigma
Product No.: B3253
F.W.: 317.2

Tris(hydroxylamine)methylamine
Supplier: VWR International
Product No.: 10315
F.W.: 121.14

6.0 PREPARATION OF REAGENTS

6.1 **0.05M Tris/HCl buffer, pH 7.4** (Reagent 1)
Dissolve 6.06g of Tris(hydroxymethyl)methylamine in approximately 800ml of analytical grade water. Adjust the pH to 7.4 (±0.05) with 5.8M Hydrochloric acid. Make up to 1000mls with analytical grade water.
Store at 2-8℃. Stable for two weeks.

6.2 **0.25mM 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB)** (Reagent 2)
Dissolve 0.1mg/ml in Reagent 1.
Store at 2-8℃. Prepare fresh daily.

6.3 **0.075M Butyrylthiocholine iodide** (Reagent 3)
Dissolve 24mg/ml in analytical grade water.
Store at 2-8℃. Stable for one week.

6.4 **Enzyme solution** (Reagent 4)
Immediately before use dissolve lyophilised enzyme at 5mg/ml in Reagent 1. Allow to stand for approximately 5 minutes. Dilute further to approximately 0.1U/ml immediately before measurement.

7.0 TEST PROCEDURE

Temperature = 25°C Wavelength = 405nm Light path = 1cm

Into plastic test tubes at 25°C pipette the following:

0.25mM DTNB (Reagent 2)	5.40ml

Incubate at 25°C for 3-4 minutes.
Before use add:

0.075M Butyrylthiocholine iodide (Reagent 3)	0.40ml

Pipette 2.9ml of this solution into the test cuvette and place the remainder of the material into the blank cuvette.
Start the reaction with the addition of 0.10ml of the enzyme solution (Reagent 4)

Record the increase in absorbance at 405nm of the test material against the blank for 3-4 minutes.
Measure the rate of increase in absorbance over the linear part of the trace. This rate will be used in the calculation.

8.0 CALCULATION

Volume activity (U/ml) = $\frac{\Delta E_{405}nm/min \times Vt \times dilution\ factor}{Vs \times \varepsilon}$

Where:

Vt = final volume of reaction mix (ml) = 3.00
Vs = sample volume (ml) = 0.10
ε = micromolar extinction coefficient for 5-thio-2-nitrobenzoic acid at 405nm = 13.3 $cm^2/\mu mole$.

Volume activity (U/ml) = ΔE_{405}nm/min x 2.26 x dilution factor

Weight activity (U/mg material) = $\frac{U/ml}{mg\ material / ml}$

Specific activity (U/mg protein) = $\frac{U/mg\ material}{mg\ protein/mg\ material}$

9.0 PROTEIN DETERMINATION

Protein is determined by the method Lowry *et al*[2] (see Procedure No, AP62, Analytical Procedures Manual).

10.0 $E^{1\%}_{280}$ DETERMINATION

The $E^{1\%}_{280}$ is determined according to Proc. No. AP63 (Analytical Procedures Manual).
Dissolve the material in Reagent 1 for E_{280} measurement (diluting in the same as required.)

11.0 REFERENCES

1. Weber H., Dtsch. Med. Wschr. (1960) **91**, 1927

2. O.H. Lowry *et al*. J. Biol. Chem. (1951), **193**, 265.