# CHOLINESTERASE

***E.C.3.1.1.8.***

***Kinetic Colorimetric Method***

*Cat.No. 101-0286* *Size 20 x 2.5 ml*

## *PRINCIPLE:*

Cholinesterase hydrolyses butyrylthiocholine to thiocholine and butyrate. Thiocholine reacts further with DNTB giving 5-thio-2nitrobenzoate, a yellow compound which can be measured at 405 nm.

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{Cholineste rase}} \text{Thiocholine} + \text{Butyrate}$$

$$\text{Thiocholine} + 5,5-\text{ditho}-\text{bis}-2\text{nitrobensoate} \longrightarrow$$
$$\longrightarrow 5-\text{thio}-2-\text{nitrobensoate}$$

## *SAMPLE:*

Serum, heparinized or EDTA plasma.

## *REAGENTS:*

1. Reagent 1 (buffer), 20x2.5 ml
   Phosphate buffer, pH 7.7 50 mmol/L

2. Reagent 2 (substrate), 1 vial with 20 tablets
   Butyrylthiocholine iodide 7 mmol/L
   5-5-dithio-bis-(nitrobenzoic acid) 0.25 mmol/L

Stable up to the expiry date when stored at +2 °C to +8 °C.

## *PREPARATION OF REAGENTS:*

Dissolve one tablet (reagent 2) with 2.5 ml of buffer (reagent 1). Gently swirl until completely dissolved. Do not shake!
This working reagent is stable 2 hours at +2 to +8 °C.

## *PROCEDURE:*

| | |
|---|---|
| Method | Kinetic (increasing) |
| Wavelength: | 405 nm (400 nm - 440 nm) |
| Cuvette: | 1 cm light path |
| Temperature: | 25 °C, 30 °C, 37 °C |
| Zero: | air or $H_2O$ |

| Pipette into tubes | 25/30 °C | 37 °C |
|---|---|---|
| Working reagent | 2500 µl | 2500 µl |
| Sample | 20 µl | - |
| Sample dil. 1:2 with saline solution | - | 20 µl |
| Mix and read initial absorbance and start stopwatch at the same time. Repeat reading after exactly 30, 60 and 90 seconds. Calculate ΔA/30 sec. | | |

## *CALCULATION:*

ΔA/30 sec x F = U/L

25/30 °C ΔA/30 s x 18950
37 °C ΔA/30 s x 37900

U/L x 16.67 = nkat/L

## *EXCEPTED VALUES:*

| Temperature | U/L | nkat/L |
|---|---|---|
| 25 °C | 3000-9300 | 50010-155031 |
| 30 °C | 3714-11513 | 61912-191922 |
| 37 °C | 4859-14443 | 81000-240765 |

## *LINEARITY:*

up to 3790 U/L ( 63179 nkat/L )

## *QUALITY CONTROL:*

| | | |
|---|---|---|
| CONTRO-N | 20 x 5 ml | Cat. No. 101-0083 |
| CONTRO-P | 20 x 5 ml | Cat. No. 101-0084 |

## *NOTE:*

If the absorbance change within 30 sec >0.200 at 405 nm, dilute the sample 1:5 with physiological solution and reassay (result x 5).

## *REFERENCE:*

1. Knedel M. and Boettger R., Klin. Wschr. 45, 325(1967) .