



CHOLINESTERASE

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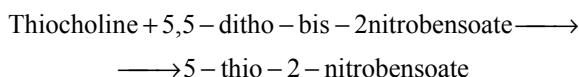
Kinetic Colorimetric Method

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.



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 ₂ O

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:

$$\Delta A/30 \text{ sec} \times F = \text{U/L}$$

$$25/30 \text{ °C} \quad \Delta A/30 \text{ s} \times 18950$$

$$37 \text{ °C} \quad \Delta A/30 \text{ s} \times 37900$$

$$\text{U/L} \times 16.67 = \text{nkat/L}$$

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) .