CHOLINESTERASE

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(Kinetic Propionylthiocholine Method)

INTENDED USE:
This reagent is intended for the *in-vitro* quantitative determination of Cholinesterase in human serum.

PRODUCT HIGHLIGHTS:
- Stability : 3 days at 2-8°C
- Linear Range : Up to 8000 U/L
- Method : Kinetic/Propionylthiocholine

CLINICAL SIGNIFICANCE:
There are two forms of cholinesterase; acetyl cholinesterase and cholinesterase or also commonly referred to as psuedocholinesterase. Acetylcholinesterase is found predominantly in erythrocytes. Cholinesterase is synthesized in the liver and is present in plasma and is the form of the enzyme routinely measured. Cholinesterase is most commonly measured as an indicator of exposure to anticholinesterases (organophosphates, including many insecticides), or inherited abnormal variants of the enzyme, which cause a decreased level of plasma cholinesterase.

Increased levels of activity may be present in nephrotic syndrome or in the recovery from liver damage.

PRINCIPLE:
Cholinesterase hydrolyses propionylthiocholine to propionic acid and thiocholine. Thiocholine reacts with 5, 5'-dithio-bis (2-nitrobenzoic acid) to form the yellow coloured 5-thio -2-nitrobenzoic acid. The rate of formation of 5-thio-2-nitrobenzoic acid, measured at 405nm, is directly proportional to cholinesterase activity in the sample. This method is a modification of the methodology of Dietz et al[2]

$$\text{Propionylthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{propionic acid} + \text{Thiocholine}$$

$$\text{Thiocholine} + \text{5, 5'-Dithio-bis (2-nitrobenzoic acid)} \longrightarrow \text{5-thio-2-nitrobenzoic acid}$$

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Propionylthiocholine	100 mmol/L
* DTNB	≥ 1mmol/L
Reagent-2	
* Buffer	100 mmol/L

pH 7.8 ± 0.1 at 25 °C
Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

	Vial / Bottle
All reagents to be stored at 2-8 °C	15x1.1 ml
1 Cholinesterase	15
2 Cholinesterase	1

Precaution: Do not ingest. Avoid contact with skin and eyes. If split thoroughly wash affected areas with water. Flush with plenty of water when disposing.

REAGENT PREPARATION:
Reconstitute the contents of each vial with the 1.1 ml of 2 Cholinesterase buffer. Mix gently until fully dissolved. DO NOT SHAKE.

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REAGENT STORAGE & STABILITY:
1. When stored between 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.
2. Working Reagent : When stored capped at 2-8°C, the reagent is stable for at least 3 days.

INDICATIONS OF REAGENT DETERIORATION :
(a) Turbidity,
(b) Absorbance> 0.8 at 405nm (1cm); and/or
(c) Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING:
Use Fresh and non-haemolysed serum.
Cholinesterase in serum is stable for 17 days when stored between 2-8°C or for 3 months when stored below - 20°C.

PROCEDURE :
- Type of reaction : Kinetic/ Increasing
- Wavelength : 405 nm
- Temperature : 30°C / 37°C
- Cuvette : 1 cm light path.
- Delay Time : 15 Seconds
- Interval : 10 Seconds
- No. of Readings : 3 Nos.
- Read Time : 30 Seconds
- Sample Volume : 20 µl
- Reagent volume : 1.0 ml
- Factor : 6899

PIPETTE INTO TEST TUBES.	TEST
• Working Reagent	1 ml
• Sample	20µl

Mix and read the first absorbance at 15 seconds and then, second reading at 45 seconds.

CALCULATIONS:
Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = Δ Abs/30 Sec x Factor

$$\text{Factor} = \frac{TV \times 1000 \times 2}{14.64 \times SV \times P}$$

Where:
TV = Total reaction volume in mL
SV = Sample volume in mL
14.64 = millimolar absorption coefficient of 5-into-2-nitrobenzoic acid at 405nm.
P = Cuvette pathlength in cm
2 = Conversion from Abs/30sec to Abs/min.

Example:
Abs/30sec = 0.150
Factor = 6899
Cholinesterase = 0.150 x 6899 = 1034 U/L

EXPECTED VALUES:
At 30°C 2618 - 6971 U/L
37°C 4900 - 11900 U/L

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

LINEARITY:

When run as recommended the assay is linear up to 8000 U/L (133.4 kat/L).

REFERENCES:

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4. Tietz NW. Textbook of Clinical Chemistry. Tietz NW (Ed) WB Saunders Company Philadelphia 1986;750.
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