CHOLINESTERASE

(Kinetic Method)

ENZOPAK

Last update 09-2020

Ref. CC1-COL.021, 20x1.1 ml

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 IU/L
- Method : Kinetic/Propionylthiocholine

DIAGNOSTIC 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.

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

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

PRESENTATION

All reagents to be stored at 2-8 °C	Vial / Bottle 20x1.1 ml
• 1 Cholinesterase	20
• 2 Cholinesterase	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Propionylthiocholine	100 mmol/L
• DTNB	≥ 1mmol/L
• Buffer	100 mmol/L

pH 7.8 ± 0.1 at 25 °C

Also contains non-reactive fillers and Stabilizers.

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.

PREPARATION OF WORKING REAGENT

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

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

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.

REACTION PARAMETERS

- Type of reaction : Two Point / Fix time / Increasing
- Wavelength : 405 nm
- Temperature : 30°C / 37°C
- Cuvette : 1 cm light path.
- Delay Time : 15 Seconds
- Interval : 30 Seconds
- Sample Volume : 20 µl
- Reagent volume : 1.0 ml
- Factor : 6899

TEST PROCEDURE

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 IU/L

NORMAL VALUES

At 30°C 2618 - 6971 IU/L
37°C 4900 - 11900 IU/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

1. Zilva JF, Pannall PR. "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. LLoyd-Luke London. 1979; pg 347.
2. Henry RJ, Clinical Chemistry Principles and Technics, New York, Harper and Row, (1974), 914-922.
3. Young Ds. Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990;3:307-308
4. Tietz NW. Textbook of Clinical Chemistry. Tietz NW (Ed) WB Saunders Company Philadelphia 1986;750.
5. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

Regd. Office.3/7, B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com Ph: +91·265·2281631
Email: mail@reckondiagnostics.com
An ISO 13485:2016 Certified Company