ALTA CALIDAD EN PRUEBAS PARA SU LABORATORIO

COD: 8002003
CHOLINESTERASE (PTC) REAGENT SET (KINETIC PROCEDURE)

INTENDED USE
Cholinesterase reagent is used for *in vitro* diagnostic use in the quantitative kinetic determination of cholinesterase in human serum, plasma, or whole blood.

INTRODUCTION
Measurement of serum cholinesterase has been used to assess liver function and monitor excessive exposure to organophosphorus insecticides.[1] It is also useful in predicting susceptibility to prolonged apnea after the administration of succinylcholine and to investigate the inheritance of variants of cholinesterase enzyme.[2,3] There are many methods for the measurement of cholinesterase activity including manometric, titrimetric and photometric procedures. The colorimetric procedure based on the Ellman reaction is sensitive and simple. Therefore, this technique forms the basis of our reagent.

PRINCIPLE
Reactions involved in the cholinesterase assay are as follows:

$$\text{Propionythiocholine} + H_2O \xrightarrow{\text{CHOE}} \text{Propionic Acid} + \text{Thiocholine}$$

$$\text{Thiocholine} + \text{DTNB} \longrightarrow \text{5-Thio-2-nitrobenzoate}$$

Cholinesterase hydrolyzes Propionythiocholine (PTC) to form Thiocholine which reacts with 5,5'-dithiobis-2-nitrobenzoic Acid (DTNB) to yield yellow 5-thio-2-nitrobenzoate with an absorbance maximum at 405 nm. Therefore, the rate of change in absorbance at 405 nm is directly proportional to cholinesterase activity.

REAGENT COMPOSITION
The Cholinesterase (PTC) Reagent, when reconstituted according to directions, has the following active ingredients. The approximate concentration of each component is as follows:

Propionythiocholine 4mM, DTNB 0.4 mM, Buffer and non-reactive stabilizers and fillers

WARNING AND PRECAUTIONS
The Cholinesterase (PTC) Reagent is for *in vitro* diagnostic use.
Normal precautions exercised in handling laboratory reagents should be followed.

REAGENT STORAGE AND STABILITY
Store the dry reagent refrigerated (2 - 8°C). Reagent is stable until the expiration date shown on the label. Reconstituted reagent is stable for 6 hours at room temperature (15 - 30°C) or for 3 days at 2 - 8°C.

REAGENT PREPARATION
TOTAL CHOLINESTERASE ACTIVITY:
Reconstitute Cholinesterase (PTC) Reagent with volume of deionized water indicated on via label. After addition of water, stopper vial and immediately mix several times by inversion.

DIBUCAINE INHIBITOR:
Reconstitute one vial of Cholinesterase (PTC) Reagent with Dibucaine Solution, instead of deionized water.

FLUORIDE INHIBITOR:
Reconstitute one vial of Cholinesterase (PTC) Reagent with fluoride solution, instead of deionized water.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Moisture has penetrated the vial and caking has occurred.
2. The reconstituted reagent has an absorbance against water greater than 1.2 at 405 nm.

SPECIMEN COLLECTION AND STORAGE
Cholinesterase activity is stable in undiluted serum for as long as 2 weeks at 2 - 8°C and up to 3 months at -20°C.[4] Serum should be removed from clot promptly.[5] EDTA does not inhibit cholinesterase activity.

Specimen preparation for measurement of cholinesterase activity in whole blood is as follows. Draw blood in tubes containing EDTA as anticoagulant. Mix whole blood thoroughly. An aliquot of blood is removed for hematocrit determination. Prepare blood hemolysate by mixing 0.1 ml blood with 1.9 ml distilled water. Mix until hemolysis is complete. Centrifuge the remaining blood to obtain clear plasma. Determine the cholinesterase activities in plasma and hemolysate by following the instructions in "Procedure" section. Samples for hemolysate cholinesterase activity are stable for 8 hours when stored at 2 - 8°C.

INTERFERING SUBSTANCES
Hemolyzed serum samples should not be used. Certain drugs and other substances are also known to affect cholinesterase activity.[5]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Spectrophotometer, with temperature controlled compartment, capable of accurately measuring absorbance at 405 nm.
2. Test tubes with optical properties suitable for use at 405 nm
3. Pipetting devices for the accurate delivery of volumes required for the assay
4. Timer
5. Dibucaine and Sodium Fluoride Solutions

AUTOMATED PROCEDURE
Refer to appropriate application manual available.

MANUAL PROCEDURE
1. Reconstitute reagent according to instructions.
2. Pipette 1.0 mL of reagent into appropriate tubes and allow to equilibrate to 30°C or 37°C.
3. Zero spectrophotometer with water at 405 nm.
4. Add 10 µL of sample (serum, plasma or hemolysate). Mix well.
5. After 15 seconds, measure the absorbance (A_1). Return tube to 30°C or 37°C for another 30 seconds and measure another absorbance (A_2).
6. Calculate the ΔA per 30 seconds by subtracting A_1 from A_2, Multiply by 2 to obtain ΔA per minute.
7. Calculate cholinesterase activity (U/L) of sample by multiplying ΔA/min. times 7426.

ALTERNATE VOLUMES
The cholinesterase activity can also be determined by using 10 µl sample and 3 mL reagent. Different calibration factors should be used if sample and reagent volumes are different from the volumes required in the procedures.

DIBUCAINE AND FLUORIDE INHIBITION ASSAYS
Following the same steps given in the Procedure, determine cholinesterase activity in the samples using Dibucaine and Fluoride containing reagents prepared according to the instructions given in Reagent Preparation. To determine percent of inhibition of activity, refer to Calculations.

CALCULATIONS (KINETIC)

$$\text{Cholinesterase Activity (U/L)} = \frac{\Delta A/\text{min.} \times TV \times 1000}{13.6 \times LP \times SV}$$

ΔA/min. = Absorbance change per min.
TV = Total Volume (1.01 mL)
SV = Sample Volume (0.01 mL)
13.6 = Millimolar absorptivity of DTNB
LP = Lightpath (1 cm)
1000 = Conversion of units per mL to Units per Liter

$$\text{Cholinesterase Activity (U/L)} = \frac{\Delta A/\text{min.} \times TV \times 1000}{13.6 \times 1.0 \times 0.01}$$

NOTE: Preparation of hemolysate involves 20-fold dilution of the sample; therefore, the Cholinesterase Activity in hemolysate calculated by the above formula should be multiplied by 20 to compensate for the dilution.

Example: $A_1 = 0.316$ $A_2 = 0.491$
$A_2 - A_1 = 0.491 - 0.316 = 0.175$
$\Delta A/\text{min.} = \Delta A/30 \text{ seconds} \times 2 = 0.350$

Cholinesterase Activity (U/L) of sample = 0.350 × 7426= 2599

DIBUCAINE AND FLUORIDE INHIBITION
Calculate percent of inhibition of Cholinesterase Activity (U/L) as follows:

$$\text{Dibucaine Inhibition of Cholinesterase Activity (\%)} = 100 - \frac{(CHE - D) \times 100}{CHE}$$

$$\text{Fluoride Inhibition of Cholinesterase Activity (\%)} = 100 - \frac{(CHE - F) \times 100}{CHE}$$

Where:
CHE= Cholinesterase Activity in sample determined by using reagent containing neither Dibucaine nor Sodium Fluoride

CHE-D= Cholinesterase Activity in sample determined by using reagent containing 0.3 mMol/L Dibucaine

CHE-F = Cholinesterase Activity in sample determined by using reagent containing 40 mMol/L Sodium Fluoride.

Erythrocyte Cholinesterase Activity:
Erythrocyte Cholinesterase Activity (ECHE) is calculated from the results obtained for plasma Erythrocyte Cholinesterase Activity (PChE), Hemolysate Cholinesterase (HChE) and the hematocrit (HCT) value of the sample.

(HChE) = (EChE x Hct*) + (PChE x (1-Hct*)

$$\text{EChE} = \frac{\text{HChE} - (\text{PChE} \times (1 - \text{Hct*})}{\text{Hct*}}$$

* Hematocrit value expressed as decimal equivalent.

LIMITATIONS
1. Extremely lipemic or icteric serum should have blank correction performed.
2. This procedure does not include Dibucaine or Fluoride for resistance studies

CALIBRATION
The procedure is calibrated by means of the millimolar absorptivity of 5-thio-2-nitrobenzoic acid, which is 13.6 at 405 rim. The linearity range of this reagent varies depending upon the sample to reagent ratio. The upper limit of linearity according to this procedure is 8,000 U/L with sample to reagent ratio of 1:100.

QUALITY CONTROL
It is recommended that high and low values of cholinesterase are included in each set of assays.
Commercially available control material with established cholinesterase values may be used for quality control.

Temperature Conversion Factor for Human Serum

	Desired Temp		
Assay Temp.	25	30	37
25	1.00	1.20	1.55
30	0.83	1.00	1.29
37	0.65	0.77	1.00

EXPECTED VALUES
The expected range of serum cholinesterase activity at 30°C was determined to be as follows:

Serum	3100 – 7700 U/L
Plasma	1700 – 4100 U/L
Whole Blood	3300 – 5500 U/L
Erythrocytes	4400 – 8200 U/L

PERFORMANCE CHARACTERISTICS
1. *Linearity:* 8000 IU/L at 30°C.
2. *Sensitivity:* An absorbance change of 0.001/min. at 405 nm corresponds to 7.4 U/L of cholinesterase activity under the stated conditions of this assay system.
3. *Comparison*: A study performed between the present procedure and one commercial product on whole blood resulted in a coefficient of correlation of 0.96 with a regression of y = 1.00 x -103 (n = 20) and on serum/plasma resulted in a coefficient of 0.99 with a regression of y = 0.96 x + 63 (n = 47).
4. *Precision Studies*:

Within Run

Mean (U/L)	S.D.	C.V.(%)
4539	173	3.8
3717	162	4.4

Run-To-Run

Mean (U/L)	S.D.	C.V.(%)
4603	153	3.3
3760	151	4.0

REFERENCES

1. Silk, E., *et al*., *Ann Clin. Biochem*. 16:57 (1979).
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