

# Infinite

## CHOLINESTERASE

### Butyrylthiocholine

#### INTRODUCTION

1. **Infinite** Cholinesterase is a reagent kit for quantitative determination of cholinesterase activity in human serum and plasma based on DGKC method using butyrylthiocholine.
2. **Infinite** Cholinesterase is a **ready-to-use, two liquid reagent system**.
3. With **Infinite** Cholinesterase, the assay is linear upto **25000 U/L**.
4. **Infinite** Cholinesterase assay can be performed in **8 minutes** at **37°C**.

#### CLINICAL SIGNIFICANCE

Cholinesterase also known as acetylcholinesterase, is found mainly in the nerve endings and in the gray matter of the brain. It is responsible for prompt hydrolysis of acetylcholine released at the nerve endings to mediate transmission of the neural impulse across the synapse. The degradation of the acetylcholine is necessary to the depolarization of the nerve so that it can be repolarized in the next conduction event.

A similar enzyme acylcholine acylhydrolase, also known as pseudocholinesterase, is found in the liver, heart, pancreas, white matter of the brain and serum, but its biological role is unknown.

Cholinesterase levels in serum are useful as a test of liver function and as an indicator of possible insecticide poisoning. Among the organic phosphorus compounds that inhibit the cholinesterase activity are many organic insecticides, such as Parathion, Sarin and tetraethyl pyrophosphate. During poisoning the level of enzyme decreases as its activity is inhibited.

#### PRINCIPLE

Cholinesterase catalyses the hydrolysis of butyrylthiocholine, forming butyrate and thiocholine, which reduces the ferricyanide ions to ferrocyanide.

The decrease in absorbance is directly proportional to the activity of cholinesterase in the specimen and is measured kinetically at 405 nm.

#### REAGENT STORAGE, STABILITY & HANDLING

The reagents R1 & R2 are ready-to-use.

The reagent kit should be stored at 2° - 8° C and is stable till the expiry date indicated on the label.

Keep the reagents away from direct light sources. Do not freeze the reagent.

Contamination of reagent should be strictly avoided.

#### COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Sodium pyrophosphate	> 70 mM
• Potassium ferricyanide	> 1 mM
• Butyrylthiocholine	> 12 mM
• Stabilizers	

#### SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Serum or EDTA/heparinized plasma can be used. Hemolysis should be avoided. Cholinesterase activity in serum/plasma is stable for 2 week 2° - 8° C.

#### PROCEDURE

- **Reaction type**.....Kinetic
- **Reaction direction**.....Decreasing
- **Wavelength**.....405 nm
- **Flowcell temperature**.....37°C
- **Zero setting with**.....Distilled water
- **Delay time**.....90 seconds
- **No. of readings**.....4
- **Interval**.....30 seconds
- **Sample volume**.....20 µl
- **R 1 volume**.....1000 µl
- **R 2 volume**.....200 µl
- **Factor**.....65800
- **Linearity**.....25000 U/L

#### Manual assay procedure :

Perform the assay as given below:

R 1 ..... 1.0 ml (1000 µl)  
Sample..... 0.020 ml (20 µl)

Mix & incubate for 5 minutes at 37°C.

R 2..... 0.200 ml (200 µl)

Mix & aspirate the assay mixture. After the initial delay of 90 seconds, record the absorbance of the test at an interval of 30 seconds for the next 90 seconds at 405 nm.

Determine the mean change in absorbance per minute and calculate the test results.

#### Calculation :

Activity of Cholinesterase in U/L =  $\Delta \text{Abs/min.} \times 65800$

#### EXPECTED VALUES

Men : 5600 - 11200 U/L

Women : 4200 - 10800 U/L

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own reference range.

#### PERFORMANCE CHARACTERISTICS

1. **Linearity limit:** If the Cholinesterase activity exceeds 25000 U/L, dilute the specimen with normal saline (0.9% NaCl) and repeat the assay. The result obtained should be then multiplied with dilution factor to obtain correct Cholinesterase activity.
2. **Detection Limit:** Values less than 432.3 U/L gives non-reproducible results.
3. **Interferences:** No interference was observed upto 500 mg/dL hemoglobin, 40 mg/dL bilirubin and 800 mg/dL lipids.

#### 4. Precision Studies:

	Within run (n = 10)			Between run (n = 20)		
	Mean (U/L)	SD (U/L)	CV%	Mean (U/L)	SD (U/L)	CV%
Sample 1	5972.9	122.8	2.1	5808.4	113.4	2.0
Sample 2	5743.8	57.5	1.0	5753.5	99.6	1.7

5. **Co-Relation Studies:** A comparison between *Infinite* Cholinesterase esterase available product gave the following co-relation(U/L):

$$y = 0.985x + 51.7$$

$$r^2 = 0.996$$









No. of samples measured: 107

### QUALITY CONTROL

To ensure adequate quality control, it is recommended that the laboratory should use a normal and an abnormal commercial reference control material. It should be realized that the use of quality control material checks both reagent and instrument functions together. Factors which might affect the performance of this test include instrument function, temperature control, cleanliness of glasswares and accuracy of pipetting.

### REFERENCES

1. Eur.J.Clin.Chem.Clin.Biochem, Vol. 30, 1992, 162-170.
2. Tietz Textbook of Clinical Chemistry, 2nd Edition, Burtis - Ashwood (1994).

	<i>In Vitro</i> Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
	Catalogue Number		Temperature Limitation
	Batch Code		Manufacturer