Cholinesterase Reagent

PRODUCT SUMMARY

Stability	:	3 days at 2-8°C
Linear Range	:	Up to 8000 U/L
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of distilled or deionized water.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		
	Consult instructions for use		Manufactured by

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

CLINICAL SIGNIFICANCE[1]
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 synthesised 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.

METHODOLOGY
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].

$$\text{Propionylthiocholine} + H_2O \xrightarrow{\textit{cholinesterase}} \text{propionic acid} + \text{thiocholine}$$

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

REAGENT COMPOSITION

Active Ingredients	Concentration
Propionylthiocholine iodide	4.0 mmol/L
5-5'Dithio-bis-(2-nitrobenzoic acid)	0.25 mmol/L
Buffer	
Also contains non-reactive ingredients	
pH 6.8	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the Cholinesterase Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

REAGENT PREPARATION
Reconstitute the contents of each vial with the volume of distilled or deionised water stated on the vial label. Mix gently until fully dissolved. DO NOT SHAKE.

STABILITY AND STORAGE
Prior to use:
When stored between 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Reconstituted Reagent:
When stored capped at 2-8°C, the reagent is stable for at least 3 days.

Indications of Reagent Deterioration:
- Turbidity,
- Absorbance > 0.8 at 405nm (1cm); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING[3]
Serum: Use non-haemolysed serum.
Storage: Cholinesterase in serum is stable for 17 days when stored between 4-23°C or for 3 months when stored below -20°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyser capable of maintaining constant temperature (30/37°C) and measuring absorbance at 405nm.
- Analyser specific consumables, eg. sample cups.
- Distilled or deionized water for reagent preparation and related equipment, eg: pipettes.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30/37°C
Wavelength	405 nm
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1:100
eg: Sample Vol	3 µL
Reagent Vol	300 µL
Delay/Lag Time	15 Seconds
Read Time	30 Seconds
Reagent Abs Limits	Low 0.00 AU
(405nm, 1cm lightpath)	High 0.80 AU
Linearity	Up to 8000 U/L
(refer to linearity section)	
Sensitivity	0.072 ΔmA/min per U/L
(405nm, 1cm lightpath)	

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

Activity in U/L = ΔAbs/min 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-thio-2-nitrobenzoic acid at 405nm (see Note 3).
P	=	Cuvette pathlength in cm
2	=	Conversion from ΔAbs/30sec to ΔAbs/min

Example:

ΔAbs/30sec	=	0.150
Factor	=	13,798
Cholinesterase	=	0.150 x 13,798 = 2070 U/L

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Valid results depend on an accurately calibrated instrument, timing, and temperature control.
3. The millimolar absorption coefficient for 5-thio-2-nitrobenzoic acid at 405 nm is 14.64.
4. Unit Conversion: U/L x 16.67 x 10^{-3} = µkat/L

CALIBRATION
Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

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QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-
- Repeat the sample controls
- If repeated results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat test with fresh reagent.
- If results are still out of control contact Technical Services or your local distributor.

LIMITATIONS
1. Young DS[4] has published a comprehensive list of drugs and substances which may interfere with this assay.
2. Grossly haemolysed samples may produce falsely elevated results.[5]
3. Avoid lipaemic and icteric samples.

EXPECTED VALUES
At 30°C 2618 - 6971 U/L (43.6 - 116.2 µkat/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[6].

PERFORMANCE DATA
The following data was obtained using the Cholinesterase Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Within Run:	LEVEL I	LEVEL II
Number of samples	20	20
Mean (U/L)	1021	3845
SD (U/L)	14.9	44.2
CV (%)	1.5	1.2

Total:	LEVEL I	LEVEL II
Number of samples	20	20
Mean (U/L)	1021	3845
SD (U/L)	24.3	81.2
CV (%)	2.4	2.1

ACCURACY
Comparison studies were carried out using a similar commercially available Cholinesterase reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	80
Range of sample results	556 - 6581 U/L
Slope	0.909
Intercept	15.8 U/L
Correlation coefficient	0.9819

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

SENSITIVITY:
When run as recommended the sensitivity of the assay is 0.072 ΔmA/min per U/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. Dietz, A.A, Rubenstein, H.M., Lubrano,T.,”Colorimetric determination of serum cholinesterase and its genetic variants by the propionylthiocholine-dithiobis (nitrobenzoic acid) procedure”. Clin.Chem. 19, No. 11, (1973);1309 - 1313.
3. Henry RJ, Clinical Chemistry Principles and Technics, New York, Harper and Row, (1974), 914-922.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990;3:307-308
5. Tietz NW. Textbook of Clinical Chemistry. Tietz NW (Ed) WB Saunders Company Philadelphia 1986;750.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

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REF

Reorder Information

Catalogue No.	**Configuration**
TR55017	20 x 7 mL