CHOLINESTERASE
Multi-Purpose (MPR)
Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	*Quantity*	*Reagent*	*Storage*
GL1001CS	4 x 40ml	Cholinesterase - 1	2-8°C
	4 x 10ml	Cholinesterase - 2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the determination of Cholinesterase in serum and plasma, on automated and semi-automated analysers.

SUMMARY AND EXPLANATION: [5]

Two related enzymes have the ability to hydrolyse acetylcholine. One is called true cholinesterase or choline esterase I, the other is called pseudo cholinesterase or choline esterase II. Choline Esterase I is responsible for the prompt hydrolysis of an acetylcholine at nerve endings to mediate transmission of the neural impulse across the synapse. The biological role of Cholinesterase II is unknown.

Measurements of Cholinesterase levels are useful for:
- Possible insecticide poisoning.
- Detection of individuals with atypical forms of the enzyme.
- Test of liver function

PRINCIPLE OF THE TEST: [1]

Cholinesterase catalyses the hydrolysis of butyrylthiocholine to thiocholine and butyrate, in presence of potassium hexacyanoferrate (III) (yellow colour). Potassium hexacyanoferrate (III) is reduced into potassium hexacyanoferrate (II) (colourless). The absorbance decrease is proportional to the cholinesterase activity in the sample.

Butyrylthiocholine + potassium Hexacyanoferrate (III) (Yellow) $\xrightarrow{\text{Cholinesterase}}$ thiocholine + butyrate + potassium Hexacyanoferrate (II) (colourless)

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Clear, pale yellow liquid.
Reagent 2: Clear, colourless liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety Precautions:
This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request.

Handling precautions:
- *Protect from direct light, contamination and evaporation.*
- *Immediately after use, recap bottles and store at 2-8°C*
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:
Instrument application procedures are available upon request.

COMPONENT COMPOSITION:

Component	*Ingredients*	*Concentration in Tests*
Reagent 1	Pyrophosphate pH 7.7	65.0 mmol/l
	Hexacyanoferrate (III)	2.0 mmol/l
Reagent 2	Goods Buffer pH 4.0	20.0 mmol/l
	Butyrylthiocholine iodide	65.0 mmol/l

REAGENT PREPARATION AND STABILITY:

Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label

Monoreagent procedure: Add 1 ml of reagent 2 to 4 ml of reagent 1.
Working solution is stable 30 days at 2-8°C.

Bireagent procedure: Liquid reagent 1 and 2 are ready for use.
Once open, components are stable until expiry date on label if stored and handled properly.

TYPE OF SPECIMEN:

Use serum, free of haemolysis, or EDTA/Heparin plasma as specimen. *Do Not Use* Citrate, Oxalate or Fluoride plasma.

It is recommended to follow CLSI procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Plasma/serum should be separated from cells as soon as possible after collection.
Stability: up to 15 days at 2-8°C.

TEST PROCEDURE:
Materials required but not supplied:

Description	*Catalog. No.*	*Description*	*Catalog. No.*
General Chemistry Calibrator	GL983	Photometer	N/A
General Chemistry Control Level 1	GL922	General Laboratory Equipment	N/A
General Chemistry Control Level 2	GL932	Saline solution 0.9gr/l NaCl	N/A

Assay procedure:

Wavelength:	λ: 405 (400 – 440) nm
Temperature:	37°C
Optical path:	1cm light path.
Reaction:	"Kinetic" (decreasing)

Bring the reagents at 15-25°C before using them. The volumes can be changed proportionally.

Monoreagent procedure: "sample starter"

	Blank	**Sample**
Working Reagent	1000µl	1000µl
Distilled water	15µl	----
Sample	----	15µl
Gently mix and Incubate for 1 minute at 37°C. Measure absorbance of reagent blank and sample then take further absorbance measurements a further 1, 2 and 3 minutes against the reagent blank. Calculate the absorbance change (ΔOD/min) per minute.		

Note: Working reagent should have an absorbance of al least 1.000 at 405nm.

Bireagent procedure: "substrate starter"

	Blank	**Sample**
Reagent 1	800µl	800µl
Distilled water	15µl	----
Sample	----	15µl
Gently mix and incubate for 1min at 37°C and add:		
Reagent 2	200µl	200µl
Gently mix and Incubate for 4 minutes at 37°C. Measure absorbance of reagent blank and sample then take further absorbance measurements a further 1, 2 and 3 minutes against the reagent blank. Calculate the absorbance change (ΔOD/min) per minute.		

Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Control results are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior to reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the Laboratory's Q.C. Programme.

CALCULATION:
Cholinesterase [U/l] = Δ OD/min x 62000. The factor and the reagent performance are related to 405nm and 37°C. (Conversion factor: Qty in µKat/l = Qty in U/l x 0.0167).

EXPECTED VALUES:

In Serum and Plasma at 37°C	*U/l*	*µKat/l*
Adults Male	5100 – 11700	85 – 195
Adults Female	4000 – 12600	69 – 210
Children till 6 months	40% to 50% higher activity than the adults	

Each laboratory should establish its own reference range. Cholinesterase results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:
Performance evaluation can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 25000 U/l (416.7 µKat/l). (Corresponding to a Δ OD/min of 0.417 at 405 nm).
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Interfering substances:
Results of study are as follows:
Bilirubin (mixed isomers): Less than 10% interference up to 300µmol/
Intralipid: I. Less than 10% interference up to 5 g/l.

Sensitivity:
The Lowest Detectable Level of Cholinesterase was estimated at 163 U/l (2.7 µKat/l).

Precision:

Within Run N = 10	Mean *(U/l)*	*SD*	*% CV*	*Between Run N = 20 for 3 days*	Mean *(U/l)*	*SD*	*% CV*
Level 1	4385	46.9	1.1	Level 1	4359	144	3.3
Level 2	4950	129	2.6	Level 2	4982	234	4.7

Method Comparison:
Using 40 samples, a comparison, between this Cholinesterase test (y) and another commercially available test (x) has given a correlation factor r = 0.9916.

BIBLIOGRAPHY:

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3. Jakobs DS. Kasten Jr BL. Demmott WR. Wolfson WL.: "Laboratory Test Handbook" Lexi-Comp and Williams & Wilkins Ed. (2nd Edition – 1990).
4. Kaplan LA. Pesce AJ.: "Clinical Chemistry", Mosby Ed. (1996).
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SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard
CE	CE Mark - Device complies with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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