Reactivos GPL

Barcelona, España

CE

- CHE -

CHOLINESTERASE

Butyrylthiocholine. Kinetic

Store at: +2+8ºC.

Presentation:
Cod. EZ006 CONT: R1 1 x 65 mL. + R2 20 x → 3 mL.

Procedure

Quantitative determination of cholinesterase (CHE).
Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

Cholinesterase hydrolysed butyrylthiocholine to butyrate and thiocholine. Thiocholine reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to form 5-mercapto-2-nitrobenzoic acid (5-MNBA) according the following reactions:

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{Butyrate} + \text{Thiocholine}$$

$$\text{Thiocholine} + \text{DTNB} \longrightarrow \text{5-MNBA}$$

The rate of 5-MNBA formation, measured photometrically, is proportional to the enzymatic activity of cholinesterase in the sample[1,2].

REAGENT COMPOSITION

R 1 Buffer	Phophate pH 7.7	50 mmol/L
R 2 Substrate	5,5-dithiobis-2-nitrobenzoic ac. (5,5 DTNB) Butyrylthiocholine	0.25 mmol/L 7 mmol/L

REAGENT PREPARATION AND STABILITY

Working reagent (WR): Dissolve (→) 1 tablet of R.2 in one vial of R.1.
Cap and mix gently to dissolve contents.
Stability: 2 hours at 2-8ºC.
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use.
Do not use reagents over the expiration date.
Do not use tablets if appears broken.

Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm. ≥ 1.20

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC,. Do not use reagents over the expiration date.

SPECIMEN

Serum or heparinized plasma[1]: Stability 7 days at 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25ºC, 30ºC or 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.

General laboratory equipment.

TEST PROCEDURE
1. Assay Conditions
- Wavelength : ..... 405 nm.
- Cuvette: ..... 1 cm light path.
- Constant temperature . . . . .25ºC / 30ºC / 37ºC.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a Cuvette:

	25ºC. – 30ºC	37ºC.
WR (mL)	1.5	1.5
Sample (µL.)	10	--
Sample dlitued ½ with NaCl 9 g/L. (µL.)	--	10

4. Mix.
5. Read the absorbance (A) of the sample, start the stopwatch and read absorbance at 1 min. interval thereafter for 1.5 min.
6. Calculate the difference of absorbance and the average absorbance difference per minute (ΔA/min.).

CALCULOS *(Nota 2)*

ΔA/min x 22710* = U/L de CHE a 25 – 30ºC.

ΔA/min x 45420* = U/L de CHE a 37ºC.

Unidades: La unidad internacional (UI) es la cantidad de enzima que convierte 1 µmol de substrato por minuto, en condiciones estándar. La concentración se expresa en unidades por litro (U/L).

Factores de conversión de temperaturas
Los resultados pueden transformarse a otras temperaturas multiplicando por:

Assay temperature	Conversion factor to		
	25ºC		25ºC
25ºC	1.00	25ºC	1.00
30ºC	0.81	30ºC	0.81
37ºC	0.64	37ºC	0.64

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control Normal Ref. QC001 and Control Pathological Ref. QC002. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES[1]

25ºC	30ºC.	37ºC
3000 - 9300 IU/L.	3714 – 11513 IU/L.	4659 – 14443 IU/L.

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Cholinesterase is an enzyme present in plasma and synthesized by the liver. Its true physiological function is unknown, so its function may be to hydrolyze choline in plasma. Cholinesterase activity is usually measured for liver function, is a sensitive test of exposure to pesticides organophosphorus and identification of patients with the atypical form of enzyme whose presents high sensitivity to succinyl-choline[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Measuring Range (at 37ºC)*:
  From detection limit of 7 U/L. to linearity limit of 9084 U/L.
  If results obtained were greater than linearity limit, dilute the sample 1/5 with NaCl 9 g/L. and multiply result by 5.
- *Precision*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	5992	3087	6277	3254
SD	70	56.1	50.5	66.0
CV (%)	1.17	1.82	0.80	2.03

- Sensitivity: 1 U/L = 0.0002 ΔA/30 seg.
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES
- Moderate haemolysis will not interfere in the results.[1,2]
- A list of drugs and other interfering substances with CHE determination has been reported by Young et. al[3,4].

NOTES
1. Use clean disposable pipette tips for its dispensation.
2. Formulation to reach constant:.

ΔA/Sec. x 22710* or 45420* = U/L CHE

$$* \frac{Tv \times 1000}{\varepsilon \times LP \times Sv}$$

Tv= Total volume in mL
ε DTNB = 13.5 at 405 nm
LP= Light path
Sv= Sample volume in mL

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