Enzytec™ D-3-Hydroxybutyric acid

Ref. No. E2610

Enzymatic assay for the determination of D-3-Hydroxybutyric acid in foodstuff and other sample materials
(33 tests in manual mode / 330 tests with automation)

Only for *in vitro* use
Store between +2 – +8 °C

Principle

The ß-hydroxybutyrate dehydrogenase (ß-HB-DH) catalyzes the oxidation of D-3-hydroxybutyrate to acetoacetate with simultaneous reduction of NAD to NADH, which is measured at 340 nm:

D-3-hydroxybutyrate + NAD^+ —— ß-HB-DH ⟶ Acetate + NADH

Reagents

#1: Buffer (Good buffer, pH> 7.5): 2 vials ~50 mL each
#2: NAD (NAD > 250 mol/L): 10 vials ~10 mL each
#3: ß-HB-DH (ß-HB-DH >50 KU/L, activators, stabilizers):
1 vial ~20 mL (ready to use)
#4: Standard (ß- hydroxybutyrate = 500 mg/L, NaN3 < 0,1%):
1 vial ~5 mL

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Preparation of working solutions

Dissolve one vial of R2 –NAD with 10 mL of R1 -BUFFER. Mix smoothly until content is dissolved. Avoid the formation of foam. Bring the reagent to room temperature before use. Close immediately after use.
Stability of the working reagent: 7 days at 2-8°C.

Sample preparation

- Use liquid, clear and nearly neutral samples directly or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- When necessary, use general sample preparation methods for enzymatic tests like water extraction, Carrez clarification, deproteinization with acids, etc…).

Test procedure

Wavelength:	340 nm
Optical path:	1 cm (glass, plastic)
Temperature:	37°C
Method:	End-point
Linearity:	up to 800 mg/l

	Reagent blank	Sample
Reagent R2-NAD	3000 µl	3000 µl
Sample / standard	-	50 µl
Distilled water	50 µl	-
Mix with a spatula and incubate at 37°C for approx. 3 min. Read absorbance A1, then add:		
ß-HB-DH (R3)	500 µl	500 µl
Mix with a spatula, incubate exactly 15 min at 37°C and read absorbance A2. Wait exactly for another 5 min and read absorbance A3		

Calculation of results

Option 1: Lambert-Beer law

Calculate the absorbance difference (ΔA) for the blank and for the samples:

- $\Delta A = (A_2 - df \times A_1) - 3 (A_3 - A_2)$
  With df (dilution factor) = dilution factor fot he absorbance because of the reagent volume added in the test:
  df = (sample + R1) / (sample + R1 + R2) = 3050/3550= 0.859
- The difference $A_3 - A_2$ represents the "creep reaction" and is subtracted from the total reaction that was measured during the first 15 min.

Subtract the reagent blank form every sample and calculate the concentration.

$\Delta A_{\text{ß-Hydroxybutyric acid}} = \Delta A_{\text{Sample}} - \Delta A_{\text{Reagent blank}}$

$c = (V \times MW \times \Delta A) / (\varepsilon \times d \times v \times 1000)$ [g/l]

c = (3.550 x 104.1 x ΔA) / (6.3 x 1.00 x 0.050 x 1000)

c = 1.1732 x ΔA [g/l] a 340 nm

With this method it is possible to use the standard (vial 4) as quality control instead as calibrator.

Option 2: calibration curve

Calculate $\Delta A_{Standard}$ for every calibration point and ΔA_{Sample} for every sample. Establish the calibration curve by using the concentration and the $\Delta A_{Standard}$ from each calibration point. The calibration curve must be repeated when changing the kit, the lot number or the calibrator. Read the ΔA_{Sample} for each sample on the calibration curve and report the concentration.

Further calculations

If the sample has been diluted, multiply the result with the dilution factor.

For solid samples tested after extraction in water:

$$\text{Content [g/100 g]} = \frac{\text{C [g/l]}}{\text{Weight}_{\text{extraction}}\ \text{[g/l]}} \times 100$$

Test performance

1. There are no interferences identified.
2. **Linearity:** the test is linear up to 800 mg/L. For higher concentrations: dilute the sample with distilled water, repeat the test and multiply the result by the dilution factor.
3. **Sensitivity** (LoD): The limit of detection which is statistically different from zero has been measured at 0,6 mg/L.
4. Applications for automated systems are available on request.

Literature

1. Methods of Enzymatic Analysis, Ed. By H.U.Bergmeyer, 3nd ed., Verlag Chemie, Weinheim, Deerfield Beach/Florid Basel (1985).
2. Council Directive (20 June 1989), Official Journal N. l212/87 (89/437/EEC)(1989)
3. Parry A.E.J. et al., J. Sci. Food Agric. 31, 905 (1980)

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