β-Hydroxybutyric Acid (D3-H) Test Kit

(Cat/No.:BC069 Size:R1:40ml×2 R2:10ml×2)

1. Reagent composition

Reagent	Specification	Composition	Storage
Reagent 1	40mL×2 bottles	matrix solution	2～8℃
Reagent 2	10mL×2 bottles	enzyme solution	2～8℃
Reagent 3	0.5mL × 1 vial	Standard (concentration see label)	2～8℃

2. Meaning of determination

This kit is used for the quantitative determination of β-hydroxybutyric acid (D3-H) content in serum. The determination of β-hydroxybutyric acid has important clinical significance for monitoring the condition of diabetic patients, early diagnosis of ketoacidosis and preventing the worsening of the condition.

3. Determination principle

β-Hydroxybutyrate (β-HB) is specifically oxidized by β-HB dehydrogenase and NAD+ to produce acetoacetic acid and NADH. Meanwhile, INT (oxidizing agent) is converted back into NAD+ and INT (reducing agent) in the presence of flavoprotein enzyme and NADH. This process is repeated cyclically. By detecting the changes in absorbance at a wavelength of 505 nm (wavelength range 470-560 nm), the content of β-HB in the sample can be quantified.

4. Steps

1.Operation of biochemical analyzer

①.Main performance parameters:

Main wavelength	505nm	Reaction method	Endpoint method	Reaction temperature	37℃
Auxiliary wavelength	700nm	Reaction direction	up	Calibration type	Linear
Sample size	10μL	Reagent 1	240μL	Reagent 2	60μL

②. Operation method:

Additives	blank	standard	Measurement
distilled water	10μL	-	-
Standard products	-	10μL	-
sample	-	-	10μL

Reagent 1	240μL	240μL	240μL
Mix well, incubate at 37°C for 5 minutes, and read the absorbance A1 at 505 nm.			
Reagent 2	60μL	60μL	60μL
Mix well, incubate at 37°C for 5 minutes, and read the absorbance A2 at 505 nm. Calculate ΔA = A2 - A1.			

The fully automatic biochemical analyzer comes with its own program parameter input method. The above basic parameters need to be combined with the fully automatic biochemical analyzer's own program parameter input method. After inputting the parameters on the machine, the reagent can be automatically measured by the instrument.

2. Spectrophotometer operation:

Additives	Blank	Standard	Sample
Double distilled water	40μL	-	-
Standard	-	40μL	-
Sample	-	-	40μL
Reagent 1	960μL	960μL	960μL
Mix well, incubate at 37°C for 5 minutes, and read the absorbance A1 at 505 nm.			
Reagent 2	240μL	240μL	240μL
Mix well, incubate at 37°C for 5 minutes, and read the absorbance A2 at 505 nm. Calculate ΔA = A2 - A1.			

5. Calculation results

$$\beta\text{ - hydroxybutyric} = \frac{\triangle A_{sample} - \triangle A_{blank}}{\triangle A_{standard} - \triangle A_{blank}} X \text{ Standard Concentration}_{(mmol/L)}$$

6. Reference value range

Normal human serum: 0.02~0.28mmol/L (it is recommended that each laboratory establish its own reference value range)

7. Product performance indicators

Reagent blank absorbance: A_{505nm}(1.0cm) ≤ 0.800;
Linear range: 0~4.5mol/L (judgment basis: r2 ≥ 0.995);
Accuracy: relative deviation ≤ 10%;
Precision: intra-batch CV＜5.0%; inter-batch relative range ≤10%.
Sensitivity: 0.2≤△A≤1.000 when testing 1.5mmol/L test object.

8. Notes

1. The ratio of sample to reagent can be adjusted according to needs.

2. Do not mix reagent 1 and reagent 2. It is not recommended to mix reagents from different batches.

3. All waste should be disposed of in accordance with local regulations. Do not use this product after its expiration date.

4. Please use the sample as soon as possible after collection. It is stable for 2 days at 2-8℃. The effect is lower after freezing.