β-Hydroxybutyric acid (β-HB) Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: BC5080

Size: 50T/24S

Components:

Reagent	Size	Storage
Reagent I	Solution 70 mL×1	2-8℃
Reagent II	Powder×2	-20℃
Reagent III	Powder×2	-20℃
Chromogenic solution	Solution 4mL×1	-20℃
Standard	Powder×1	2-8℃

Solution preparation:

1. **Reagent II:** Add 1.5mL distilled water to each Reagent II before use. Mix thoroughly. It can be stored at -20℃ for three weeks after dispensing to avoid repeated freezing and thawing.
2. **Reagent III:** Add 400μL distilled water to each Reagent III before use. Mix thoroughly. It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing.
3. **Working Solution:** Reagent I, Reagent II and Reagent III are mixed by the ratio of 850μL:40μL:10μL （1T） to make working solution according to sample number. Mix thoroughly. Keep it at 37℃ for 15 min **(this step can't be omitted)**. The working solution should be **used up in 4 hours**.
4. **Standard: S**odium 3-hydroxybutyrate. Add 980μL distilled water before use. Mix thoroughly. The 8 mg/mL of sodium 3-hydroxybutyrate standard solution could be stored at -20℃ for four weeks.

Product Description：

β-Hydroxybutyric acid (β-HB), in patients with severe acidosis, NADH production increases due to acidosis, which in turn promotes the ratio of β-hydroxybutyric acid to acetoacetic acid to increase from the normal 2:1 to 16: 1. β-hydroxybutyric acid is of great significance in the diagnosis and treatment of diabetic ketoacidosis. It is also of great significance for the early diagnosis of diabetes. **The kit is suitable for serum, plasma, urine and other liquid samples.**

At pH8.8 and 37℃, β-hydroxybutyrate reacts under the catalysis of β-hydroxybutyrate dehydrogenase, and NAD^+ is oxidized to NADH. In the presence of 1-mPMS, WST-1 can react with NADH to produce water-soluble formazan with a characteristic absorption peak at 450nm. The content of β-HB can be calculated by detecting the wavelength change at 450nm.

Reagents and Equipment Required but Not Provided：

Spectrophotometer, desk centrifuge, pipette, 1mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure

I. Sample preparation:

Serum, plasma, urine or other liquid samples: Detect sample directly. If the solution is turbid, perform the measurement after centrifuging.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 450nm, set zero with distilled water.
2. Standard working solution: Dilute 8mg/mL sodium 3-hydroxybutyrate standard solution with distilled water to 0.125、0.0625、0.03125、0.015625、0.0078125mg/mL standard solution before use.
3. Determination:

Reagent（μL）	Test tube	Contrast tube	Blank tube	Standard tube
Sample	100	100	-	-
Distilled water	-	-	100	-
Standard solution	-	-	-	100
Working solution	900	-	900	900
Reagent I	-	900	-	-
React at 37℃ for 10min.				
Chromogenic solution	50	50	50	50
React at 37℃ for 20min. (Light avoidance)				
Measure absorbance at 450nm. Record as A_T、A_C、A_B、A_S. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$. Blank tube and standard curve only need to be tested one or twice.				

III. Calculations:

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding ΔA standard is y-axis. Then the linear regression equation y＝kx+b is obtained. Bring ΔA_T into the equation to get x (mg/mL).

2. Calculate

(1) Calculate by protein concentration

β-HB content (μmol/mg prot)=$x\times V_S\div(V_S\times Cpr)\div126.09\times1000=7.931x\div Cpr$

(2) Calculate by volume

β-HB content (μmol/mL)=$x\times V_S\div V_S\div126.09\times1000=7.931x$

V_S: Sample volume, 100μL=0.1mL;

V_E: Extract solution volume, 1mL;

Cpr: Protein concentration of the sample, mg/mL.

126.09: Relative molecular mass of sodium 3-hydroxybutyrate, mg/mmol;

1000: Unit conversion factor, 1 mmol=1000 μmol.

Note:

1. After color development, please complete the test within 10 minutes.

2. If the measured absorbance value is lower or higher than the linear range absorbance value. The sample can be added or diluted before determination.

Examples:

1. Take 10μL bovine serum to test, follow the determination procedure to operate. Determination with 1mL glass cuvette, and calculate $\Delta A_T=A_T-A_C=0.585-0.057=0.528$, standard curve: y=0.7905x+0.0221, calculate x=0.640, according with volume of sample to calculate:

β-HB content (μmol/mL) =7.931x=5.076 μmol /mL

Related products

BC0710/BC0715 α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit
BC2150/BC2155 Citric Acid (CA) Content Assay Kit
BC0950/BC0955 Succinate Dehydrogenase (SDH) Activity Assay Kit
BC0380/BC0385 Pyruvate Dehydrogenase (PDH) Activity Assay Kit
BC2160/BC2165 Isocitrate Dehydrogenase Mitochondrial (ICDHm) Activity Assay Kit