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

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC5085

Size: 100T/48S

Components:

Reagent	Size	Storage
Reagent I	Solution 25 mL×1	4℃
Reagent II	Powder×2	-20℃
Reagent III	Powder×2	-20℃
Chromogenic solution	Solution 1.5mL×1	-20℃
Standard	Powder×1	4℃

Solution preparation:

1. Reagent II: Take one powder and add 600μL distilled water before use. Mix thoroughly. Unused reagents should be store at -20℃ for three weeks. Avoid repeated freezing and thawing.
2. Reagent III: Take one powder and add 400μL distilled water before use(about 100T). Mix thoroughly. Unused reagents should be store at -20℃ for two weeks. Avoid repeated freezing and thawing. Reagent III is not easy to save, so give one more powder.
3. Working Solution: According to the ratio of 85:4:1, Reagent I, Reagent II and Reagent III are mixed into working solution before use. According to the test requirements. 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: Sodium 3-hydroxybutyrate. Add 980μL distilled water before use. Mix thoroughly. That is 8mg/mL of sodium 3-hydroxybutyrate standard solution.

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/microplate reader, desk centrifuge, pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, 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/microplate reader for 30min, adjust wavelength to 450nm, set zero with distilled water.

2. 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	20	20		
Distilled water			20	
Standard solution				20
Working solution	180		180	180
Reagent I		180		
React at 37℃ for 10min.				
Chromogenic solution	10	10	10	10
React at 37℃ for 20min. (Light avoidance)				
Take 200μL to 96 well flat-bottom plate or micro glass cuvette. Measure absorbance at 450nm. Record as A_T、A_C、A_B、A_S. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$.				

Note: blank tube and standard curve only need to be test one or two times.

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 into the equation to get x (μmol/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, 20μL=0.02mL;

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 20μL bovine serum to test, follow the determination procedure to operate. Determination with 96 well flat-bottom plate, and calculate $\Delta A_T=A_T-A_C=0.455-0.082=0.373$, standard curve: y=0.4913x+0.0043, calculate x=0.750, according with mass of sample to calculate:

β-HB content (μmol/mL) =7.931x=5.948 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