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# 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:** AK0005

**Size**: 100T/48S

**Components:**

| Reagent | Size | Storage |
|---|---|---|
| Extract solution | Solution 110 mL×1 | 4℃ |
| Reagent I | Solution 20 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: 8mg sodium 3-hydroxybutyrate. Add 960μL distilled water before use. Mix thoroughly. That is 80μmol/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 80μmol/mL sodium 3-hydroxybutyrate standard solution with distilled water to 1.25, 0.625, 0.3125, 0. 15625, 0.078125μmol/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 III | | 180 | | |
| React at 37℃ for 10min. | | | | |
| Chromogenic solution | 10 | 10 | 10 | 10 |
| React at 37℃ for 20min. | | | | |
| 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)=$x\times V_S\div(V_S\times Cpr)=x\div Cpr$

(2) Calculate by volume

β-HB content (μmol/mL Serum (plasma) or urine)=$x\times V_S\div V_S=x$

$V_S$: Sample volume, 20μL=0.02mL;

$V_E$: Extract solution volume, 1mL;

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

**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) =x=0.750μmol/mL.

**Related products**

AK0282/AK0281 α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit
AK0400/AK0399 Citric Acid (CA) Content Assay Kit
AK0504/AK0503 Succinate Dehydrogenase (SDH) Activity Assay Kit
AK0554/AK0553 Pyruvate Dehydrogenase (PDH) Activity Assay Kit
AK0249/AK0248 Isocitrate Dehydrogenase Mitochondrial (ICDHm) Activity Assay Kit