# **Blood Calcium Content Assay Kit**

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

**Operation Equipment:** Spectrophotometer/Microplate reader

**Cat No:** NA0569 **Size:** 100T/96S

## **Components:**

Reagent I: 5 mL×1, store at 4°C. Reagent II: 5 mL×1, store at 4°C.

Reagent III: Liquid×1, (blank bottle, self-provided). Add 9 mL of absolute methanol and 1 mL of acetone to a 15 mL reagent bottle, mix thoroughly.

Standard: 0.5 mL×1, 2 μmol/mL CaCl<sub>2</sub>•2H<sub>2</sub>O, store at 4°C. Dilute 5 times when the solution will be used to prepare as 0.4 μmol/mL standard.

# **Description:**

Blood calcium is almost present in plasma, so blood calcium mainly refers to plasma calcium which contain ionized calcium and bound calcium. The ionized calcium plays a physiological role directly. It is in dynamic balance with the bound calcium and affected by PH in blood. Blood calcium is related with many physiological function, too high or too low can affect normal physiological function. The kit is used for detecting free calcium concentration of blood.

In the strong alkaline solution, free calcium react with GBHA to form red calcium -GBHA compound which has an absorption peak at 520 nm; Free calcium concentration is calculated according to detect the absorbance at 520 nm.

# Required but not provided:

Adjustable pipette, Spectrophotometer/Microplate reader, micro glass cuvette/96 well plate, absolute methanol, acetone and distilled water.

#### **Protocol:**

- 1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 520 nm, set zero with distilled water.
- 2. Add samples according to the table:

| Reagent Name (μL)    | Blank tube (B) | Standard tube (S) | Test tube (T) |
|----------------------|----------------|-------------------|---------------|
| Serum                | -              | -                 | 12            |
| Distilled water      | 12             | -                 | -             |
| 0.4 μmol/mL Standard | -              | 12                | -             |
| Reagent I            | 50             | 50                | 50            |
| Reagent II           | 50             | 50                | 50            |

| Reagent III | 100 | 100 | 100 |
|-------------|-----|-----|-----|

Note: Mix thoroughly, detect the absorbance A of 520 nm after incubating for 5 minutes, record  $A_T$ ,  $A_B$ ,  $A_C$ .

#### **Calculation:**

Blood Calcium(
$$\mu$$
mol/dL)= [C<sub>S</sub>×(A<sub>T</sub>-A<sub>B</sub>)÷(A<sub>S</sub>-A<sub>B</sub>)]×100  
=40×(A<sub>T</sub>-A<sub>B</sub>)÷(A<sub>S</sub>-A<sub>B</sub>)

C<sub>S</sub>: 0.4 μmol/mL; 100: 1 dL=100 mL

#### Note:

- 1. It is advisable to take blood on an empty stomach in the morning and complete the measurement as soon as possible after taking blood.
- 2. Try to complete the measurement within 10min.
- 3. Since the reaction needs to be determined as soon as possible, when using a micro cuvette, it is recommended to measure 5-10 samples per batch.
- 4. If A<sub>T</sub> more than 0.8, suggest dilute with distilled water before detecting.

## **Experimental example:**

1. The mouse plasma is taken and operated according to the determination steps.  $A_T$ =0.296,  $A_B$ =0.092, and  $A_S$ = 0.306

Blood calcium content ( $\mu$ mol/dL) =  $40 \times (A_T - A_B) \div (A_S - A_B) = 38.131 \ \mu$ mol/dL.

#### **Recent Product Citations:**

[1] Cao W, Su Y, Liu N, et al. Role of Ca<sup>2+</sup> in Inhibiting Ischemia-Induced Apoptosis of Parathyroid Gland Cells in New Zealand White Rabbits[J]. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 2020, 26: e920546-1.

#### **Related Products:**

NA0669/NA0428 Blood Potassium Content Assay Kit NA0668/NA0427 Blood Magnesium Content Assay Kit NA0737/NA0426 Blood Phosphate Content Assay Kit NA0667/NA0425 Blood Sodium Content Assay Kit

### **Technical Specifications:**

The detection limit: 0.008 μmol/mL The linear range: 0.025-1.5 μmol/mL