ELK Biotechnology

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Calcium Assay Kit Instruction

Catalog No.:BC019 Microplate Method

I. Principle of Measurement

Calcium (Ca^{2+}) ion reacts with methyl thymol blue complexon (MTB) in an alkaline condition to give blue complex product. The absorbance at certain wavelength which is proportional to the blue complex concentration can be measured spectrophotometrically and thus the calcium concentration can be calculated.

II. Reagent Compositions and Preparation

Reagent I: MTB Solution, 1 Bottle×10 ml. Preserved at 4°C without light struck.
Reagent II: Alkaline Solution, 1 Bottle×20 ml. Preserved at RT.
Reagent III: 1 Bottle×1 ml. Preserved at RT. Were the temperature too low, the crystallization may occur and under such circumstance, warm the solution till clear solution generated prior to use.
Reagent IV: 2.5mM Calcium Solution, 1 Bottle×1 ml. Preserved at 4°C without light struck.
All reagents are stable for 6 months under the desirable conditions.
Preparation of Reagent IV Solution (1mM): Dilute the 2.5mM calcium solution with deionized water at the desirable rate.
Preparation of working fluid I (serum/plasma): Mix reagent I and II with the ratio of 1:2 prior to the measurement.
Preparation of working fluid II (tissue): Mix reagent I, II and III with the ratio of 10:20:1 prior to the measurement.

III. Sample Requirement

1. Samples should be collected with common practice. Samples can be serum, plasma, , tissue homogenate, cultured cells or culture medium.
2. Samples are stable at 2-8°C for 3-4 days and for a couple of weeks at -20°C.

IV. Procedures

1. Serum/Plasma

Compositions (μ l)	Blank	Standard	Sample
Deionized Water	10		
Reagent IV (1mM)		10	
Serum Sample			10
Working Fluid I	250	250	250

Mix thoroughly and set aside for 5 min. Record the optical density (OD) at 610 nm with a plate reader.

2. Tissue

Pretreatment: Weigh the tissue sample precisely and add deionized water with the ratio of 1 g tissue to 9 ml water. Homogenize in an ice water bath and centrifuge the homogenate at 2,500 rpm for 10 min. Extract the supernatant.

Compositions (μl)	Blank	Standard	Sample
Deionized Water	10		
Reagent IV		10	
Homogenate Supernatant			10
Working Fluid II	250	250	250

Mix thoroughly and set aside for 5 min. Record the optical density (OD) at 610 nm.

V. Calculation Formula and Example

1. Serum Sample

$$\frac{Ca^{2+}Conc.}{mM} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1\ mM} \times CoD$$

Note: CoD represents the coefficient of dilution in the sample pretreatment process.

Example: Rat serum was diluted to 2.5 times of its initial volume and measured with the OD values equal to 0.2428, 0.3918 and 0.3699 respectively.

$$\underset{mM}{Ca^{2+}Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{1mM}{C_{Standard}} \times CoD = \frac{0.3699 - 0.2428}{0.3918 - 0.2428} \times 1 \times 2.5$$
$$= 2.1332mM$$

Example: Canine serum was diluted to 2.5 times of its initial volume and measured with the OD values equal to 0.2428, 0.3918 and 0.3902 respectively.

$$\underset{mM}{Ca^{2+}Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{1mM}{C_{Standard}} \times CoD = \frac{0.3902 - 0.2428}{0.3918 - 0.2428} \times 1 \times 2.5$$
$$= 2.4727mM$$

Example: Cell supernatant was diluted to 2.5 times of its initial volume and measured with the OD values equal to 0.2428, 0.3918 and 0.3639 respectively.

$$\underset{mM}{Ca^{2+}Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{1mM}{C_{Standard}} \times CoD = \frac{0.3639 - 0.2428}{0.3918 - 0.2428} \times 1 \times 2.5$$
$$= 2.019mM$$

2. Tissue Sample

$$\underset{mmol/g}{Ca^{2+}Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{1\ mM}{C_{Standard}} \div \underset{g/L}{C_{Protein}}$$

Example: 10% rat brain tissue homogenate was prepared and measured with the OD values equal to 0.2428, 0.3918 and 0.2698 respectively. The protein concentration of the homogenate was 3.8475 g/L.

$$\underset{mmol/g}{Ca^{2+}Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \underset{1mM}{C_{Standard}} \div \underset{g/L}{C_{Protein}} = \frac{0.32698 - 0.2428}{0.3918 - 0.2428} \times 1 \div 3.8475$$
$$= 4.5528 \times 10^{-2} mmol/g$$

VI. Notes

1. To avoid calcium contamination, it is recommended to use disposable 96-well microplates which is available by the Institute.
2. Hemolysis, fats in blood may cause the interference of the results along with the blood taken from patients suffering jaundice.
3. During the homogenization process, deionized water should be used instead of physiological saline to avoid calcium contamination.
4. This assay kit is designed for the measurement on automatic/semiautomatic biochemical analyzer.
5. This protein concentration is recommended to be measured with the total protein assay kit by the Institute. (A045-2 and A045-3).
6. This assay kit is designed for scientific research only.