Calcium Microplate Assay Kit

User Manual

Catalog # CAK1105

(Version 1.2D)

Detection and Quantification of Calcium (Ca^{2+}) Content in Serum, Urine, Saliva and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Calcium is essential for all living organisms, where Ca^{2+} sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes. 99% of calcium is found in bones and teeth with the remaining 1% found in the blood and soft tissue. Serum calcium levels are tightly controlled (8.4-11.4 mg/dL) and any variation outside this range can have serious effects. Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. Calcium ion channels control the migration of calcium ions across cell membranes, permitting the activation and inhibition of a wide variety of enzymes. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels that can occur in severe alcoholism. In humans, when the blood plasma ionized calcium level rises above its set point, the thyroid gland releases calcitonin, causing the plasma ionized calcium level to return to normal. When it falls below that set point, the parathyroid glands release parathyroid hormone (PTH), causing the plasma calcium level to rise.

The calcium ions can react with MTB. The products can be measured at a colorimetric readout at 612 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	10 ml x 1	4 °C
Masking Reagent	Powder x 1	4 °C
Masking Reagent Diluent	1.5 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard (3 mmol/L)	1 ml x 1	4 °C
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Note:

Dye Reagent: add 8 ml distilled water to dissolve before use.

Masking Reagent: add 1 ml Masking Reagent Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 612 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For serum sample

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Blank	Standard	Sample
Distilled water	10 µl	--	--
Standard	--	10 µl	--
Sample	--	--	10 µl
Reaction Buffer	100 µl	100 µl	100 µl
Masking Reagent	10 µl	10 µl	10 µl
Dye Reagent	80 µl	80 µl	80 µl
Mix, wait for 5 minutes, measured at 612 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the serum sample

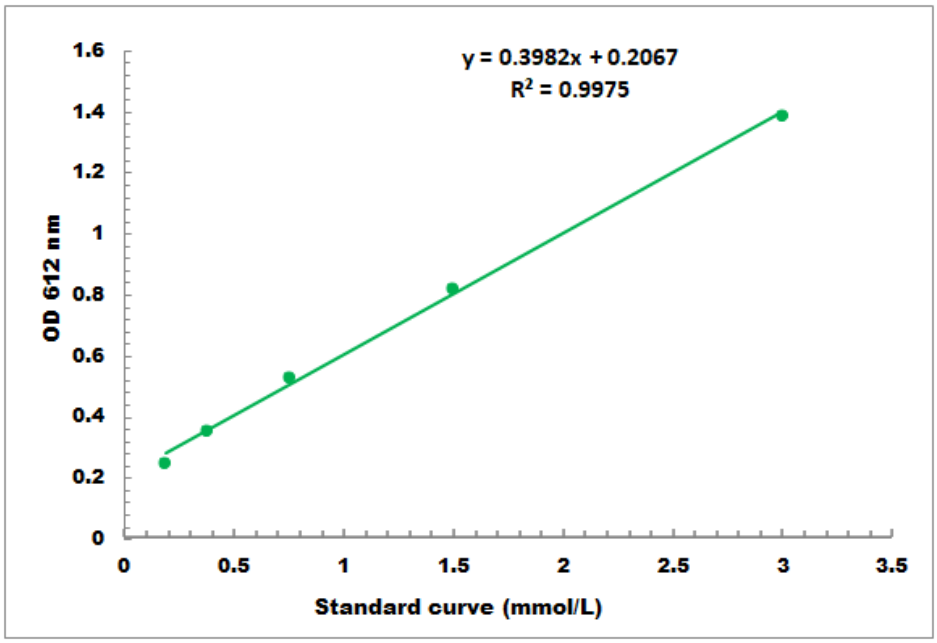
Ca^{2+} (mmol/L) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

= 3 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

$C_{Standard}$: the concentration of standard, 3 mmol/L.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 3 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES