MANUAL

Calcium Assay Kit (Colorimetric)

Catalog #: MA-CA

ISO 13485

Introduction

Calcium, the most abundant mineral in the human body, plays a pivotal role as a crucial intracellular element, regulating many cellular processes. Calcium is found in either the free ion form or in bound complexes, such as the calcium phosphate and calcium carbonate complexes constituting bone tissue. Calcium signaling governs various physiological processes, including muscle contraction, cell adhesion, release of hormones/neurotransmitters, glycogen metabolism, cell proliferation/differentiation, blood clotting, nerve or sympathetic impulse transmission, and structural support of the skeleton. Disruptions in the integrity of cell-specific calcium signaling systems are associated with certain human diseases.

RayBio® Calcium Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool for measuring calcium concentration in plasma, serum, cell lysates, urine and other biological liquid samples. In this assay, Calcium reacts with Arsenazo in a slightly alkaline medium forming a purple-colored complex with absorbance at 650 nm. The color intensity is proportional to the calcium concentration in the sample.

Storage

The entire kit may be stored at room temperature for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	RT
Calcium Standard	1 vial (100 µl of 10 mg/dL)	RT
Calcium Assay Solution	15 ml	RT

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 650 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate or oxalate and mix by inversion (**Do not use EDTA**!). Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, Calcium levels in human plasma are in the range of 8.9-10.4 mg/dL. Plasma samples can be diluted at least 1:4 with Sample Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, Calcium levels in human serum are in the range of 8.9-10.4 mg/dL. Plasma samples can be diluted at least 1:4 with Sample Buffer.

4. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. Urine samples can be used directly, or diluted with Sample Buffer.

NOTE:
Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 mg/dL – 3 mg/dL (see Table below),

1. Label 7 microtubes #1 through 7 which with the following concentrations: 3, 1.5, 0.75, 0.375, 0.1875, 0.09375, 0 mg/dL.
2. Pipette 70 µL of Sample Buffer into labeled tube #1, and 50 µL Sample Buffer into labeled tubes #2 – tube #7.
3. Pipette 30 µL of 10 mg/dL Calcium Standard (provided) into tube #1 to make 3 mg/dL Calcium Standard.
4. To make the 1.5 mg/dL standard, pipette 50µL of the tube #1 into the tube labeled #2. Mix thoroughly.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 50 µl of the prior concentration until the 0.09375 mg/dL is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh, mixed thoroughly and used immediately.

Labeled Tubes	Calcium Standard (µL)	Sample Buffer (µL)	Standard Conc. (mg/dL)
1	30µL of 10mg/dL Stock	70µL	3
2	50µL of Tube #1	50µL	1.5
3	50µL of Tube #2	50µL	0.75
4	50µL of Tube #3	50µL	0.375
5	50µL of Tube #4	50µL	0.1875
6	50µL of Tube #5	50µL	0.09375
7	0µL	50µL	0

Assay Procedure

Each Calcium standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 10 µL of the diluted Calcium standards or samples to the 96-well microtiter plate.
2. Initiate the reaction by adding 150 µL of Calcium Assay Solution to each well.
3. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
4. Incubate the plate for 5 minutes at room temperature.
5. Measure the absorbance at 650nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 mg/dL) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 650nm as a function of Calcium concentration.

Determination of sample Calcium concentration

Calcium (mg/dL) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

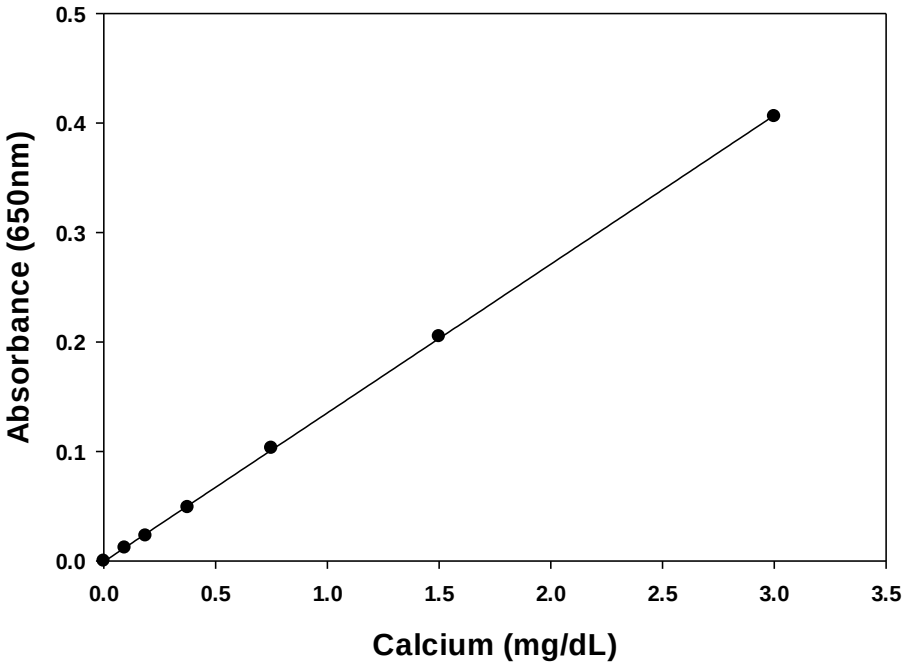
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 mg/dL)

Slope is from the plot of Calcium concentration vs. Absorbance shown in Typical data below

DF = Sample Dilution factor (DF = 1 for undiluted samples)

Note: If the calculated Calcium concentration of the sample is higher than 3 mg/dL, dilute the sample in Sample Buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable concentration of Calcium is about 0.048 mg/dL.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 2.5%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 6.9%.

This product is for research use only.