
Product Manual

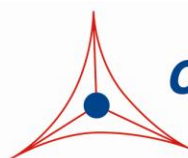
Calcium Assay Kit

Catalog Number

MET-5121

250 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.

Creating Solutions for Life Science Research

Introduction

The chemical element calcium is a reactive earth metal that is essential for all life. Calcium plays critical physiological and biochemical roles in the mammalian cell. Calcium is a second messenger in signal transduction pathways such as in G-protein coupled receptor signaling. Calcium is released into neurons through voltage sensitive calcium channels at the presynaptic membrane in response to an action potential and ultimately signals neurotransmitter release from neurons. In heart cells, a cardiac action potential results in a prolonged membrane depolarization due to the action of slower calcium channels opening and holding the membrane voltage near their equilibrium potential. In muscle cells, the sarcoplasmic reticulum releases a store of calcium ions when the cell is stimulated: the released calcium ions then enable the cross-bridge muscle contraction cycle. Calcium also acts as a cofactor for many enzymes, plays an important role in fertilization at the level of oocyte maturation as well as egg activation, and regulates the levels of several hormone levels that are important for proper bone formation.

Cell Biolabs' Calcium Assay Kit is a simple colorimetric assay that measures the total amount of free calcium present in biological samples in a 96-well microtiter plate format. Calcium forms a complex with the colorimetric probe (containing phthalein purple) to form an intermediate which is then measured with a standard 96-well spectrophotometric plate reader. Samples are compared to a known concentration of calcium standard within the 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 250 assays, including blanks, calcium standards, and unknown samples. Sample calcium concentrations are determined by comparison with a known calcium standard. The kit has a detection sensitivity limit of 15.6 μM calcium.

Related Products

1. STA-500: cAMP ELISA Kit (Colorimetric)
2. STA-501: cAMP ELISA Kit (Chemiluminescent)
3. STA-440: 96-Well Ras Activation ELISA Kit (Colorimetric)
4. STA-441: 96-Well Ras Activation ELISA Kit (Chemiluminescent)
5. STA-674: Glutamate Assay Kit
6. MET-5090: Adenosine Assay Kit

Kit Components

1. Calcium Standard (Part No. 51211A): One 100 μL vial at 500 mM.
2. Assay Buffer (Part No. 51212B): One 13 mL bottle.
3. Colorimetric Probe (Part No. 51213B): One 25 mL bottle.

Materials Not Supplied

1. Distilled or deionized water
2. Microcentrifuge tubes
3. 10 μL to 1000 μL adjustable single channel micropipettes with disposable tips

4. 50 μL to 300 μL adjustable multichannel micropipette with disposable tips
5. Standard 96-well clear microtiter plate
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 570 nm

Storage

Upon receipt, store the kit at 4°C.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in deionized water and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in deionized water .
- Cell lysates: Resuspend cells at $1-2 \times 10^6$ cells/mL in deionized water. Homogenize or sonicate the cells on ice. Centrifuge to remove debris at 18000 xg for 15 minutes at 4°C. Cell lysates may be assayed undiluted or diluted as necessary in deionized water.
- Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary in deionized water.
- Serum or Plasma: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary in deionized water.

Note: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

Preparation of Standard Curve

Prepare fresh Calcium Standards before use by diluting in deionized water according to Table 2 below.

Standard Tubes	500 mM Calcium Solution (μL)	Deionized Water (μL)	Calcium (μM)
1	2	998	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 2. Preparation of Calcium Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 μL of each Calcium Standard or unknown sample into wells of a 96-well microtiter plate.

3. Add 50 μL of Assay Buffer to each well. Mix the well contents thoroughly.
4. Add 100 μL of Colorimetric probe to each well. Mix the well contents thoroughly.
5. Incubate the reaction plate for 5-10 minutes protected from light at room temperature.
6. Read the plate at 570 nm using a microplate spectrophotometer.
7. Subtract the blank standard value from the other standard and unknown values to obtain the net OD 570 nm values.

Example of Results

The following figures demonstrate typical Calcium Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

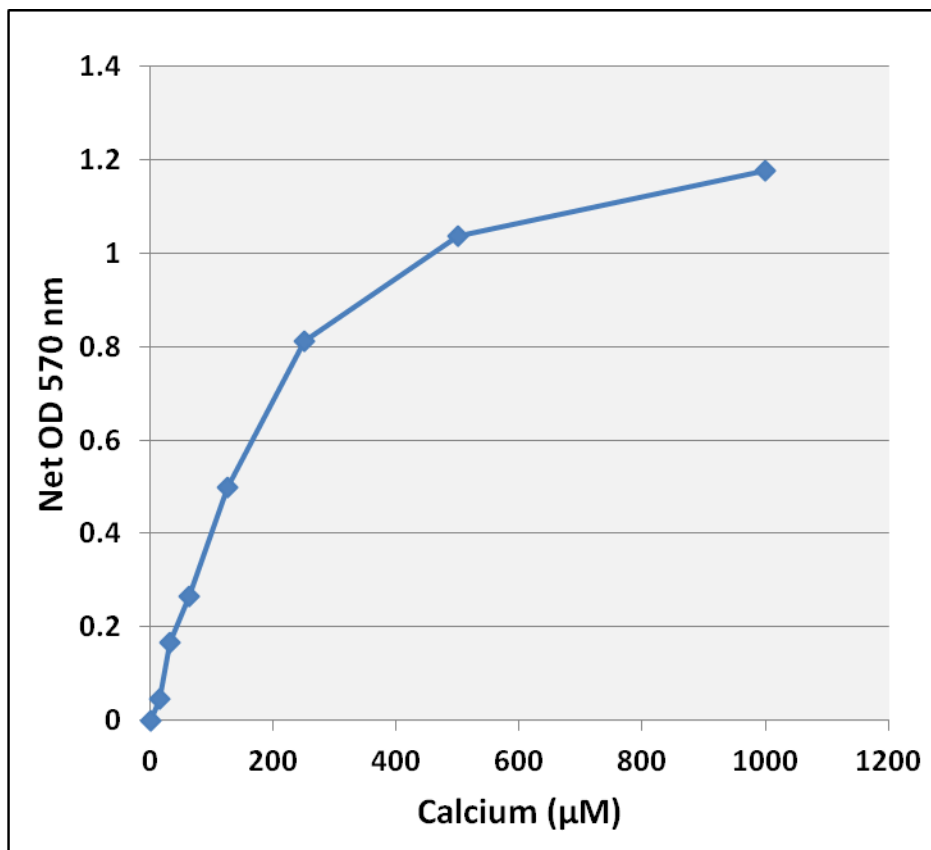


Figure 2: Calcium Standard Curve.

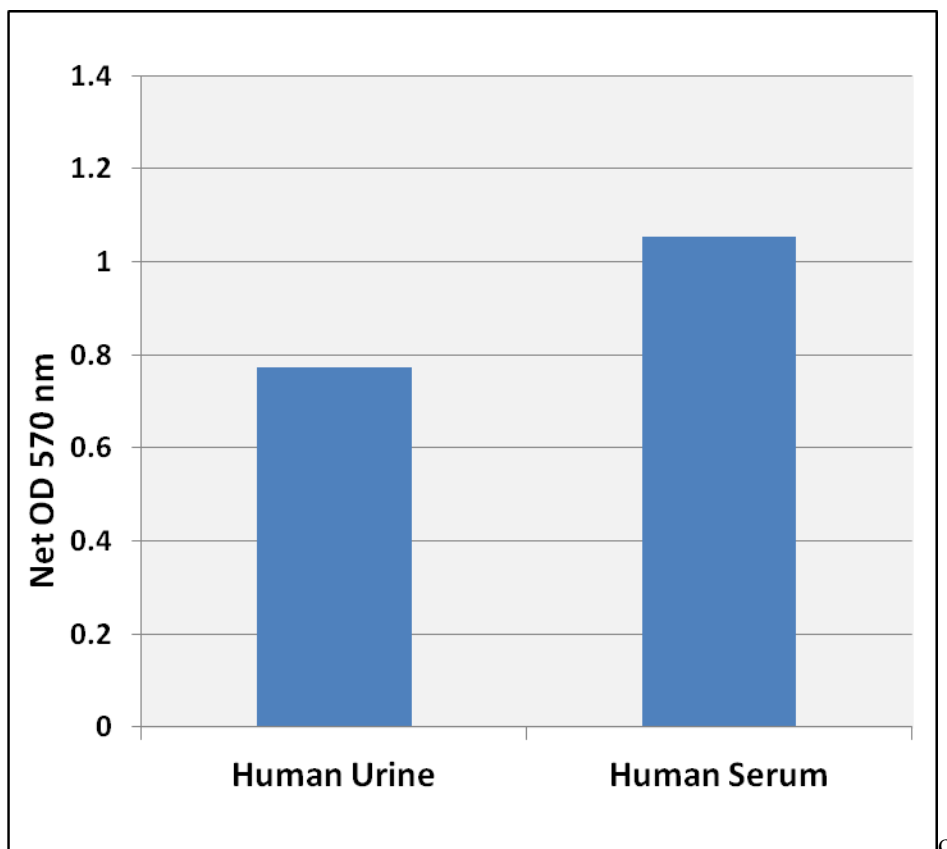


Figure 3: Calcium Detection in Normal Human Urine or Serum. Calcium was detected in human samples. Samples were tested undiluted according to the Assay Protocol.

References

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Recent Product Citation

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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