Product Information

Calcium Colorimetric Assay Kit

I. Kit Contents:

Components	K2067-250 250 assays	Cap Color	Part Number
Calcium Assay Buffer	15 ml	WM	K2067-C-1
Chromogenic Reagent	25 ml	NM	K2067-C-2
Calcium Standard (500 mM)	100 µl	Yellow	K2067-C-3

II. Introduction:

Calcium plays important roles in many cellular processes and is essential for all living organisms. 99% of calcium is present in bones and teeth with the remaining 1% in the blood and soft tissue. Calcium is involved in mediating the constriction and relaxation of blood vessels, muscle contraction, hormone secretion and nerve impulse transmission. Calcium ion channels regulate the migration of calcium ions across cell membranes, permitting the inhibition and activation of a variety of enzymes. Vitamin D deficiency, chronic kidney failure and low blood magnesium levels can cause low calcium levels, which can have serious effects. Serum calcium levels are tightly controlled at 8.4 - 11.4 mg/dL.

The Calcium Colorimetric Assay Kit provides a simple and fast way for detection of calcium concentration in various biological samples based on colorimetric method. The chromogenic complex (λ = 575 nm) formed between calcium ions and 0-cresolphthalein can be easily qualified using a microtiter plate reader or a spectrophotometer at 575 nm. The assay can detect the physiological range of calcium concentration 0.4-100 mg/dL (0.1 - 25 mM).

III. Reagent Preparation and Storage Conditions:

The kit as supplied is stable for 1 year from the shipping date under proper storage conditions. Calcium Assay Buffer and Chromogenic Reagent are ready to use as supplied. Store at 4℃ when not in use. Warm to room temperature before use. Protect from light.

IV. Calcium Assay Protocol:

1. Standard Curve Preparations: Dilute the Calcium Standard to 5 mM (20 mg/dL) by adding 10 µl of the 500 mM Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells to give 0, 0.4, 0.8, 1.2, 1.6, 2.0 µg calcium per well. Bring the volume to total 50 µl with dH_2O.

2. Sample Preparation: Serum or urine samples can be used directly in this assay. Place 10 µl samples in wells in a 96-well plate. For other liquid samples, add 2 - 50 µl sample into individual well. Bring the total volume to total 50 µl with dH_2O. Samples can be assayed without any prior treatment. Some MRI contrast agents can cause transient interference in this assay.

3. Additions:

A) Add 90 µl of the Chromogenic Reagent to each well containing standards, samples or controls and mix gently.

B) Add 60 µl of the Calcium Assay Buffer to each well and mix gently.

4. Incubate the reaction for 5 - 10 minutes at room temperature. Protect from light.

5. Measure the OD at 575 nm. The chromophore is unstable and will fade slightly over time, so read the standard and samples within 30 minutes.

6. Calculations: Correct background by subtracting the value derived from the 0 Calcium control from all sample and standard readings (Note: The background reading may be significant and must be subtracted from sample readings). Plot standard curve µg/well vs. $O.D_{575\ nm}$ readings. Then apply the sample readings to the standard curve to get Calcium sample amount in the wells (Sa). The Calcium concentrations in the test samples:

C = Sa/Sv (µg/µl or mg/ml)

Where: Sa is the Calcium Sample Amount (in µg) from standard curve.

Sv is the Sample Volume (µl) added into the sample well.

Calcium molecular weight: 40.

Calcium concentration in your sample can be expressed as mg/ml, mg/dL or mM (mmol/liter).

1 mg/ml = 100 mg/dL; 1 mM = 4 mg/dL.

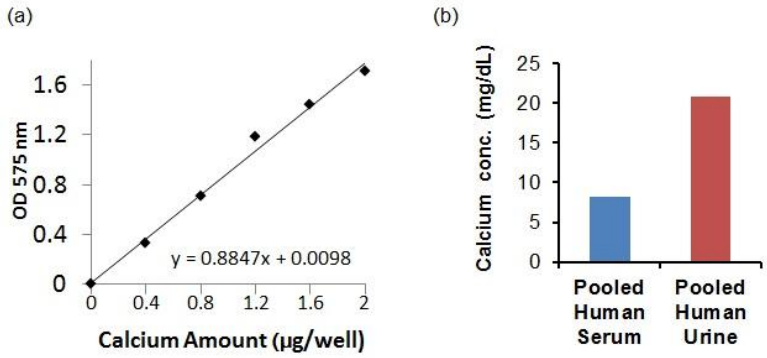

Figure: (a) Standard Curve. (b) Quantification of Calcium from pooled human serum (10 µl, 1:1 diluted) and pooled human urine (10 µl, 1:1 diluted). Assay was performed following the kit protocol.

V. Frequently Asked Questions

1. Which anticoagulants do not interfere with Calcium assay?

Heparin is the only anticoagulant that does not interfere

2. What about hemoglobin/hemolysis?

Assay will work with ≤ 750 mg/ml hemaglobin

3. The protocol recommends using 2x10^6 cells in 500 µL of buffer, however I only have 50,000 - 150,000 cells per sample. Each test only requires 2 - 50 µL of samples however. Could the sample preparation be scaled down, i.e. use 50 µL of buffer with 2x10^5 cells?

Yes, surely it can be scaled down. Our only concern is that if she is making samples in just 50 µl, you will not have enough for the pilot expt and duplicates for the main expt. Maybe you can have more cells and volume for just one sample to do the pilot expt and determine if 50 µl homogenate is enough for duplicates in the main expt. But in a nutshell, you can scale down the volumes to suit your needs as long as you still do the final expt properly.

4. I want to meassure the CA+2 in neutrophils. You have a specific buffer for their preparation (lysis)?

You can use the Calcium assay buffer provided with the kit. Use 2 - 5 x 10^6 cells, homogenize with the buffer, centrifuge and take the sup for the assay.

5. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

6. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

7. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

8. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

9. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

10. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

11. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

12. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

13. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, It is recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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