Rev. 1

ScienCell™
Research Laboratories

Calcium Assay (CA)

Catalog #8128,
250 tests in 96-well plate

Product Description

As an essential mineral for all living organisms, 99% of calcium is deposited in bones and teeth, while 1% found in extracellular fluid or within cells bound to proteins. Changed level of calcium in body fluid, such as serum, is found to be associated with many diseases such as hyperparathyroidism, neoplastic diseases, hypoparathyroidism, nephrosis and etc. ScienCell™ Calcium Assay (CA) provides a simple and direct colorimetric measurement of calcium concentration in biological samples, utilizing the purple-red complex formed between calcium and ortho-cresolphthalein in alkaline medium. The optimized formulation eliminates the interference from magnesium and helps to release calcium bound to proteins. Intensity of the developed color at 570 nm is proportional to the calcium concentration in the physiologically important range (0.3-20 mM).

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8128a	1	Calcium Standard (20 mM)	0.5 ml	4ºC
8128b	1	AMP Buffer	12.5 ml	4ºC, dark
8128c	1	Color Reagent	12.5 ml	4ºC, dark

Material Supplied by User

Microplate reader (measuring absorbance at OD 570 nm)
96-well clear plates
Phosphate-buffered saline (PBS)

Product Use

This kit is suitable for measuring calcium concentration in plasma, serum, cell lysates, urine and other biological liquid samples. CA is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures

Quality Control

CA is applied to calcium standards serially diluted from 20 to 0.3125 mM, and linearity can be proved, as shown in Figure 1.

Shipping

All components are shipped on gel ice.

Procedure (96-well plate)

A. Preparation of working standards:

1. Obtain 8 test tubes and label them #1 through #8. Add 20 µl of DI H_2O into tubes #2 through #8.
2. Add 20 µl of the 20 mM calcium standard into tube #1.
3. Add 20 µl of the 20 mM calcium standard into tube #2 and mix well to get the 10 mM calcium standard.
4. Transfer 20 µl of the 10 mM calcium standard from tube 2 to tube 3 and mix well to get the 5 mM calcium standard.
5. Repeat step 3 for tubes 4-7 to serially dilute the calcium standards. Do not add calcium standard to tube 8, which serves as the blank.
6. Obtain a 96-well plate and prepare 3 replicates of each calcium standard by aliquoting 5 µl/well of each calcium standard into triplicate wells of the 96-well plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7	#8
A	20 mM	10 mM	5 mM	2.5 mM	1.25 mM	0.625 mM	0.313 mM	Blank
B	20 mM	10 mM	5 mM	2.5 mM	1.25 mM	0.625 mM	0.313 mM	Blank
C	20 mM	10 mM	5 mM	2.5 mM	1.25 mM	0.625 mM	0.313 mM	Blank

B. Sample preparation

1. **Cell lysates**:
   For adherent cells, carefully remove the culture supernatant and wash the cells twice with cold 1X PBS. For suspension cells, centrifuge at 1,000 x g for 10 minutes to pellet the cells, then discard the supernatant and ensure no PBS remains before adding the lysis buffer. Resuspend the cells in lysis buffer with protease inhibitors at a concentration of $2x10^7$ cells/ml, pipetting gently to mix. Incubate the lysates with gentle rocking at 2–8 °C for 30 minutes. Transfer the lysate to microcentrifuge tubes and centrifuge at 14,000 x g for 10 minutes. It is advisable to measure protein concentration in the samples using a total protein assay. Use the lysates immediately, or aliquot them and store at -70 °C for future use. If using thawed lysates, keep them on ice until needed.

2. **Plasma Samples:**
   Collect blood using an anticoagulant like citrate or oxalate (avoid EDTA) and mix by gently inverting the tube. Centrifuge at 1,000 x g for 10 minutes at 4°C. Carefully collect the plasma supernatant without disturbing the buffy coat. Test samples immediately or store them at -80°C for later use. Plasma samples may be diluted at least 1:4 with Sample Buffer.

3. **Serum**:
   Draw blood into a tube without anticoagulant and allow it to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes. Carefully remove the serum supernatant without disturbing the buffy coat. Test the samples immediately or store them at -80°C. Serum samples can also be diluted at least 1:4 with Sample Buffer.

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

C. Working reagent and measurements:

1. For each well of 96-well plate, mix 50 µl of AMP Buffer with 50 µl of Color Reagent. Prepare enough working reagent based on the total number of standards and samples to be measured. The working reagent is stable for 24 hours at room temperature.
2. Add 95 µl of working reagent to each well containing 5 µl of sample or calcium standard, incubate for 15 minutes at room temperature.
3. Read absorbance at 570 nm.

D. Calculations:

1. Average the calibrated absorbance values (OD_{570nm}) of each sample, calcium standard and blank wells.
2. Correct background by subtracting the average OD_{570nm} of blank from the average OD_{570nm} of each sample and calcium standard.
3. Generate the standard curve by plotting the calibrated OD_{570nm} of the calcium standards against the calcium concentrations, as shown in Figure 1.
4. Determine the calcium concentration of each sample based on the standard curve.

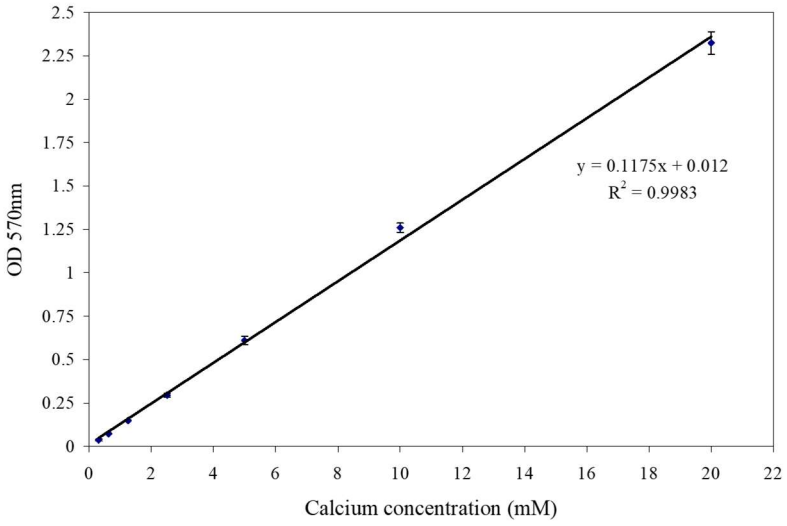

Figure 1. A typical calcium standard curve measured by ScienCell™ Calcium Assay.