Calcium Assay
(CA)
Cat. No. 8128, 250 tests

Introduction

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 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 (λ=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)	1 ml	4ºC
8128b	1	AMP Buffer	50 ml	4ºC, dark
8128c	1	Color Reagent	50 ml	4ºC, dark

Quality Control

The ScienCell™ Calcium Assay is applied to calcium standards serially diluted from 20 to 0.3125 mM, and linearity can be proved, as shown in Figure 1.

Procedures

A. Preparation of working standards:

1. Obtain 8 test tubes and label them A through H. Add 30 µl of DI H_2O into tubes B through H.
2. Add 30 µl of the 20 mM calcium standard into tube A.
3. Add 30 µl of the 20 mM calcium standard into tube B and mix well to get the 10 mM calcium standard.
4. Transfer 30 µl of the 10 mM calcium standard from tube B to tube C and mix well to get the 5 mM calcium standard.
5. Repeat step 3 for tubes D-G to serially dilute the calcium standards. Do not add calcium standard to tube H, which serves as the blank.
6. Obtain a 48-well plate and prepare 3 replicates of each calcium standard by aliquoting 10 µl/well of each calcium standard into triplicate wells of the 48-well plate, according to the plate format shown in Table 1.

B. Preparation of working reagent:

1. For each well of 48-well plate, mix 200 µl of AMP Buffer with 200 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.

C. Assay Procedure:

1. Add 400 µl of working reagent to each well containing 10 µl of sample or calcium standard, incubate for 15 minutes at room temperature.
2. 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.

		#1	#2	#3	#4	#5	#6	#7	#8
calcium standard	**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
samples	**D**								
	E								
	F								

Table 1. Plate format of calcium standards and samples in calcium assay.

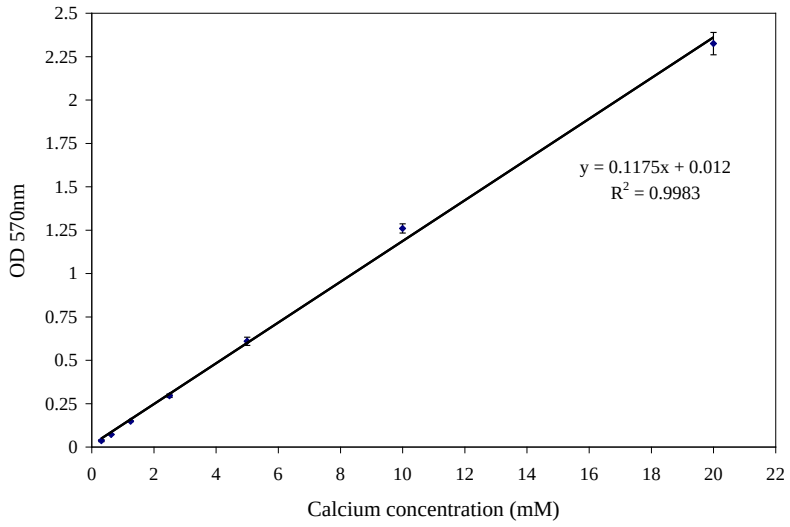

Figure 1. A typical calcium standard curve measured by ScienCell™ Calcium Assay.