MA-0104

Calcium Assay

(100 wells, Colorimetric, OD 575 nm, Store at room temperature)

Background Information:

Calcium (MW 40.078 g/mol) is one of the most important metal ions in biochemistry and is essential for all living organisms. Ca^{2+} functions as a second messenger in many systems, through its sequestration and release into the cytoplasm via a variety of different types of calcium channels. The calcium channels control the flow of calcium ions across cell membranes, resulting in the activation and inhibition of a wide variety of enzymes. Plasma calcium levels are tightly regulated with the normal range of 8.5-10.2 mg/dL with levels below 7.6 or above 16 considered extreme. In the Calcium Assay, Ca^{2+} binds to alkaline O-Cresolphthalein Complexone resulting in a chromophore with λ_{max} = 575 nm). Possible interference by Magnesium is prevented by the presence of 8-Hydroxyquinoline. AkrivisBio's Calcium Assay provides a simple direct measurement of Ca^{2+} in a variety of samples with a useful range of 0 – 1 µg.

Assay Principle:

1 – Calcium and other ions are solubilized in Assay Buffer. Mg^{2+} is chelated to remove interference.
2 – Calcium ligand O-Cresolphthalein is added to form the chromophore

Assay Components:

Assay Buffer	15 ml	WM	MA-0104A
Chromogenic Reagent	25 ml	NM	MA-0104B
Calcium Standard	400 µl	Yellow	MA-0104C

Storage and Handling:

Store at room temperature. All reagents are ready to use as supplied.

Assay Protocol:

1. Calcium Standard Curve:

Vortex Calcium Standard then centrifuge briefly before opening. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl into a series of wells in a 96-well plate to give 0 - 0.4 - 0.8 - 1.2 - 1.6 - 2.0 µg calcium per well. Adjust the well volume to 50 µl with DI H_2O.

2. Sample Test:

Serum or urine samples may be used directly. Apply 10 µl of each sample to wells in a 96-well plate. Other samples will require 2 – 50 µl depending on the amount of Ca^{2+} expected. Adjust the total volume of all wells to 50 µl with DI H_2O. No pretreatment of samples is necessary.

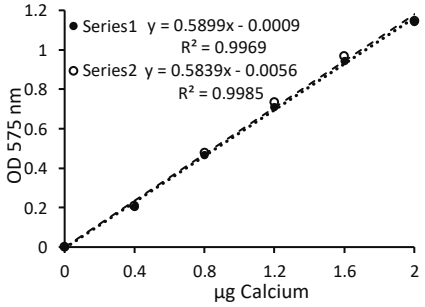
3. Color Development:

Add 90 µl of the Chromogenic Reagent to each well containing standards, samples or controls and mix gently. Add 60 µl of Assay Buffer to each well and mix gently. Allow full color development to proceed for 5-10 minutes.

4. Measure the OD at 575 nm within 15 – 30 minutes. The chromophore is somewhat unstable and will fade slightly over time.

5. Typical Result:

	Standard Raw Values		Background Corrected Values	
µg Standard	OD			
0	0.1178	0.1187	0	0
0.4	0.3235	0.3280	0.2057	0.2093
0.8	0.5844	0.5969	0.4666	0.4782
1.2	0.8279	0.8520	0.7101	0.7333
1.6	1.0628	1.0853	0.9450	0.9666
2.0	1.2604	1.2650	1.1426	1.1463



Calcium binds Cresolphthalein at either a 1:1 or 1:2 ratio. The 1:1 ratio has dramatically less absorbance than the 1:2 ratio which can be seen by the noticeable nonlinearity of the standard curve above about 1.5 µg of Ca^{2+}. The upper end of the linear range can be extended by increasing the reagent concentration, but at the expense of losing linearity near zero. We have optimized the Cresolphthalein concentration to preserve linearity in the most useful range of concentrations. If samples are seen to give results in the higher part of the standard curve, we advise repeating the assay with half of the initial amount if higher precision in the true amount of calcium is required.

6. Calculations:

Subtract the 0 µg Calcium reading from all other readings. Plot the Calcium standard curve. The slope of the line defines the OD/µg of Calcium. Divide the background corrected sample values by the slope of the standard curve to determine the amount of calcium in the sample wells. To convert back to calcium in the original samples:

A – Divide the µg calcium in the test wells by the volume of sample added to the well = µg calcium per µl of sample
B – Multiply the µg calcium per µl of sample by any dilution factor used during sample preparation = µg calcium per µl of original sample.

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