

ABS_Bio™ Calcium Detection Kit (Cat# K301-200; 200 assays; store kit at 4°C)

Introduction

Calcium is an essential mineral for all living organisms, 99% of calcium is found in bones and teeth with the remaining 1% found in the blood and soft tissue. Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. Abnormal calcium levels have been tied to neurological, endocrine, cardiovascular, digestive, metabolic, and pulmonary diseases, as well as cancer.

The ABS_Bio™ Calcium Detection Kit provides a simple, sensitive, one-step colorimetric assay to detect Ca^{2+} in plasma, serum, urine, saliva and cell (tissue) homogenates. In this assay, methylthymol blue dye, in alkaline condition rapidly and sensitively forms a highly specific blue complex with calcium. The intensity of color, measured at 610 nm (600-620 nm), is directly proportional to the calcium in the sample. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay, linear detection range of 0.08-10 mg/dL (20-2500 μM). It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (200 tests)

Assay Diluent: 10 mL Calcium Standard (100 mg/dL): 2 mL Reagent A: 25 mL Reagent B: 25 mL
Storage and Handling: Store kit at 4°C. Shelf Life: 12 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, Plasma (do not use citrate, EDTA as anticoagulant), Urine or environment liquid samples can be measured directly, sample should be diluted with assay diluent to ensure the readings are within the standard curve range. Homogenize Cell (2×10^6) or tissue (20 mg) sample in 200 μL cold PBS or assay diluent. Centrifuge to collect the supernatant. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C. Transfer 10 μL sample into the clear 96-well flat bottom plate in duplicate.

2. Standard Preparation

Transfer 20 μL 100 mg/dL calcium standard into 180 μL assay diluent to get 10 mg/dL calcium standard, then following the table to generate 10, 6, 3, 1 and 0 mg/dL Ca^{2+} standards.

Calcium std(μL)	Assay diluent(μL)	final calcium concentration (mg/dL)
50	0	5
30	20	3
15	35	1.5
5	45	0.5
0	50	0

Transfer 10 μL of appropriate standards into the 96-well plate in duplicate. The blank control containing assay diluent only.

3. Reaction

Prepare enough working reagent by mixing 100 μL Reagent A and 100 μL Reagent B for each reaction. Transfer 200 μL prepared working reagent into each standard and samples well. Tap plate to mix well. Incubate 5 min. at room temperature, protected from light.

4. Measurement

Read the optical density at 610 nm (600-620 nm).

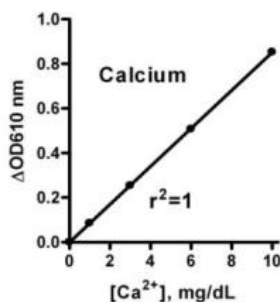
5. Calculation

Average the duplicate OD610 nm reading for standard and sample. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the calcium concentration of the standards. Determine the slope and calculate the calcium concentration of samples.

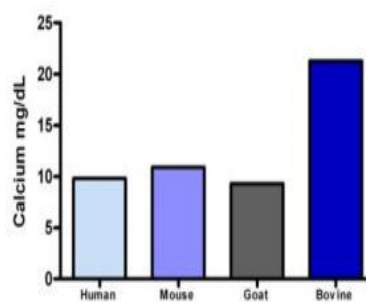
$$[\text{Calcium}] = (\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}) / \text{Slope} \times n \text{ (mg/dL)}$$

$\text{OD}_{\text{sample}}$ and OD_{blank} are related optical density of the sample and assay buffer. n is the sample dilution factor.

Typical Standard Curve



Ca²⁺ standard in 96 wells-plate assay (colorimetric).



Serum samples in 96 wells-plate assay.

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.08 mg/dL (20 μ M), and linear detection range up to 10 mg/dL (2500 μ M) in 96-well plate colorimetric assay. Sensitivity was determined as 1 μ M. Samples with values above 10 mg/dL (2500 μ M) should be dilute with assay diluent, re-assayed, and multiply results by dilution factor.

Interferences

The assay is highly specific for Ca^{2+} , interferences from magnesium, iron, lipid, protein are minimized. For prepare plasma sample, do not use citrate or EDTA as anticoagulant. Heparin is recommend.

References

Zerwekh, J.E. et al. 1984, Clin. Chem. 30:452-453
Mezei, L.M. et al. 1983, United States Patent. #4382122
Glindler, E.M. et al. 1972, Am J Clin Pathol. 58:376-382

Related Products:

ALP Colorimetric Detection Kit (#K109-200)
ALP Fluorimetric Detection Kit (#K110-200)
ALP Substrate Solution (#C8040)
BCIP/NBT Substrate system (#C8041)
Creatinine Detection Kit (#K148-200)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
HRP Substrate system (#C8042)
Xanthine Colorimetric Detection Kit (#K133-100)
SOD Activity Detection Kit (#K129-100)
Ammonia Colorimetric Detection Kit (#K203-100)
Sulfate Detection Kit (#K149-200)
Pyruvate Detection Kit (#K150-100)

Heme Detection Kit (#K169-200)
Lactate Detection Kit (#K207-100)
NAD/NADH Detection Kit (#K120-100)
NADP/NADPH Detection Kit (#K123-100)
Nitric Oxide Detection Kit (#K116-100)
Phosphate Detection Kit (#K198-500)
Urea Detection Kit (#K158-200)
pNPP substrate solution (#C8071)
TBARS Detection Kit (#K145-100)
Peroxidase Activity Detection Kit (#K126-200)
Hemoglobin Detection Kit (#K168-200)
Xanthine Oxidase Activity Detection Kit (#K132-100)
Lactate Dehydrogenase Activity Detection Kit (#K107-100)
Alcohol Dehydrogenase Activity Detection Kit (#K113-100)
Formaldehyde Detection Kit (#K118-100)