

Calcium Assay Kit (Fluorometric)

6/18

(Catalog # K409-100; 100 assays; Store at -20°C)

I. Introduction:

Calcium (Ca, Atomic Number: 40), is an essential element for all living organisms. In human, Calcium ion (Ca^{2+}) is released from bone to bloodstream and its level in serum is tightly controlled (8.4-11.4 mg/dL). Ca^{2+} acts as second messenger and plays many biological roles in signal transduction, gene expression, muscle contraction, synaptic plasticity and hormone release. Recent studies have found that dysregulation of calcium homeostasis leads to health problems, such as Alzheimer's Diseases and Parkinson's Disease, etc. BioVision's Calcium Fluorometric Assay kit provides an easy and quantitative method to measure Calcium in various biological fluids. Low concentration of *N,N,N',N'*-tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) is included in the assay and used to pre-treat samples to eliminate interference from other di-valent metals such as Zinc. The assay is based on the ability of our proprietary probe that binds specifically to Calcium cations generating a high fluorescence signal. The fluorescence is directly proportional to the amount of calcium, which can be quantified by measuring the generated fluorescence (Ex/Em= 500/530 nm) using a microplate reader. This assay kit provides an add-and-read, non-radioactive, and high-throughput adaptable assay suitable for Calcium detection. The assay is rapid (less than 10 min) and sensitive (Limit of Detection: <0.1 μM).

II. Applications:

- Measurement of Calcium concentration in biological samples
- Analysis of the effects of effectors/synthetic agents/stimuli on Calcium signaling

III. Sample Type:

- Biological Fluids: Serum, Plasma, etc.

IV. Kit Contents:

Components	K409-100	Cap Code	Part Number
Calcium Assay Buffer	50 ml	NM	K409-100-1
Calcium Probe	2 vials	Clear	K409-100-2
TPEN (1000X)	20 μl	Brown	K409-100-3
Calcium Standard (500 mM)	100 μl	Yellow	K409-100-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom for Fluorometric measurement
- Multichannel or single channel Pipettes
- Multi-well spectrophotometer (Fluorescence reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Warm Calcium Assay Buffer to room temperature (RT) before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Calcium Probe:** Reconstitute each vial with 110 μl dH₂O and vortex until fully dissolved. Store at 4°C while in use. For long-term storage, aliquot, and store at -20°C. Avoid freeze/thaw.
- **TPEN (in DMSO) and Calcium Standard (500 mM):** Store at 4°C or -20°C. Bring to room temperature (RT) before use. For long-term storage, store at -20°C.

VII. Calcium Fluorometric Assay Protocol:

1. Sample Preparation: Prior to assay, serum, plasma samples (about 200 μl) should be boiled at 95°C for 5 min, cool down at room temperature for 5 min, spin down at 10,000 x g for 10 min at room temperature, and collect the supernatant for assay. Dilute the samples in 1:50 with Calcium Assay buffer. Add 1-10 μl samples into a 96-well white plate. Adjust final volume to 45 μl with Calcium Assay Buffer. Take 10 μl of TPEN (1000X) into 490 μl Calcium Assay Buffer to make diluted TPEN (20X). Add 5 μl of diluted TPEN (20X) to each sample, mix well and incubate at room temperature for 5 min to remove possible interferences.

Notes:

- Calcium concentration can vary over a range. Human Serum (Normal ranges): 8.4 – 11.4 mg/dL.
 - For unknown samples, we suggest to test several doses to ensure the reading are within the standard curve.
 - Avoid use the EDTA-treated samples (plasma and/or serum).
- 2. Calcium Standard Curve:** Dilute the Calcium Standard to 1 mM by adding 2 μl of 500 mM Calcium Standard to 998 μl dH₂O, mix well. Dilute further to 25 μM (25 pmol/ μl) Calcium Standard by adding 10 μl of 1 mM Calcium Standard to 390 μl dH₂O. Mix well. Add 0, 2, 4, 6, 8, 10 μl of the 25 μM Calcium Standard into a 96 well clear plate to generate 0, 50, 100, 150, 200, 250 pmol/well of Calcium standard. Adjust volume to 50 μl /well with Calcium Assay Buffer.
- 3. Reaction Mix:** Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 50 μl Reaction Mix containing:

	Reaction Mix
Calcium Assay Buffer	48 μl
Calcium Probe	2 μl

Add 50 μl of the Reaction Mix to each well containing the Standard and test samples. Mix well.

4. Measurement: Gently shake the plate for 5 min. on a shaker at room temperature protected from light. Measure the fluorescence using a microtiter plate reader at Ex/Em= 500/530 nm (with Cut-off).

5. Calculation: Subtract the 0 standard reading from all standard and sample readings. Plot the Calcium Standard Curve. Determine Calcium amount (S_a) based on the Calcium Standard Curve. The Calcium concentrations in the sample can be calculated as follow:

$$C = S_a / S_v \times D = \text{pmol}/\mu\text{l} = \mu\text{M}$$

Where: S_a = the amount of Calcium (pmol) from the calculation above

S_v = the sample volume added into reaction well (μl)

D = Sample Dilution Factor

Calcium Atomic Mass: 40 g/mol

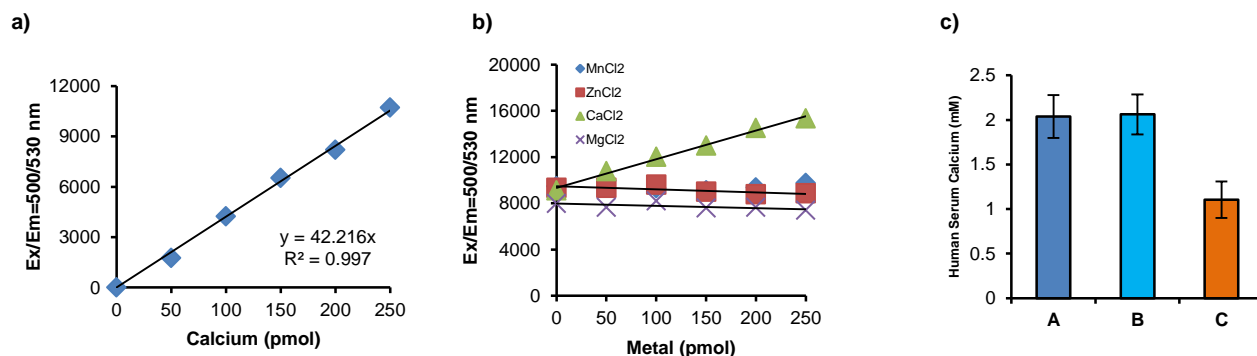


Figure: (a) Calcium Standard Curve. (b) Assay Specificity: Calcium and other Di- and poly-valent metals (Manganese, Zinc, Magnesium) were tested to evaluate possible interferences. After treatment with TPEN, the interferences from other metals were completely considerably reduced. (C) Measurement of Calcium in three Human Normal Pooled Serum samples (1:50 dilution; 2 μl for measurement). Assays were performed according to the kit protocols.

VIII. RELATED PRODUCTS

Zinc Colorimetric Assay Kit (K387)
 Calcium Colorimetric Assay Kit (K380)
 Cobalt Colorimetric Assay Kit (K505)
 Magnesium Colorimetric Assay Kit (K385)
 Phosphate Assay Kit (Fluorometric) (K420)
 Total Sulfite Assay Kit (Colorimetric) (K699)
 Ascorbic Acid Quantification Kit (K661)
 Glucose Assay Kit II (K686)
 Lactate Assay Kit (K627)
 Phosphate Assay Kits (K420)
 Hemin Assay Kit (K672)
 L-amino Acid Assay Kit (K639)
 Glutathione Detection Kits (K264)

Ammonia Colorimetric Assay Kit (K370)
 Chloride Colorimetric Assay Kit (K530)
 Iron Colorimetric Assay Kit (K390)
 Nickel Colorimetric Assay Kit (K510)
 Sodium Assay Kit (Colorimetric) (K391)
 ADP/ATP Ratio Assay Kit (K255)
 Fatty Acid Assay Kit (K612)
 Uric Acid Assay Kit (K608)
 Pyruvate Assay Kit (K609)
 Triglyceride Assay Kit (K622)
 Glycogen Assay Kit II (K648)
 ADP Colorimetric Assay Kit II (K356)

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