Calcium Colorimetric Assay Kit (BN00641)

(Catalog BN00641; 250 assays; Store kit at 4° C)

I. Introduction:

Calcium is essential for all living organisms, where Ca^{2+} sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes. 99% of calcium is found in bones and teeth with the remaining 1% found in the blood and soft tissue. Serum calcium levels are tightly controlled (8.4-11.4 mg/dL) and any variation outside this range can have serious effects. Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. Calcium ion channels control the migration of calcium ions across cell membranes, permitting the activation and inhibition of a wide variety of enzymes. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels that can occur in severe alcoholism. Assay Genie's Colorimetric Calcium Assay Kit utilizes the chromogenic complex (λ = 575 nm) formed between calcium ions and 0-cresolphthalein to provide a simple assay in the physiologically important range of calcium concentration 0.4-100 mg/dL (0.1-25 mM).

II. Kit Contents:

Components	250 assays	Cap Color
Calcium Assay Buffer	15 ml	WM
Chromogenic Reagent	25 ml	NM
Calcium Standard (500 mM)	100 µl	Yellow

III. Reagent Preparation and Storage Conditions:

The kit as supplied is stable for 1 year from the shipping date under proper storage conditions. Calcium Assay Buffer and Chromogenic Reagent are ready to use as supplied. Store at 4° C when not in use. Warm to room temperature before use. Protect from light.

IV. Calcium Assay Protocol:

1. **Standard Curve Preparations:** Dilute the Calcium Standard to 5 mM (20 mg/dL) by adding 10 µl of the 500 mM Standard to 990 µl of dH2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells to give 0, 0.4, 0.8, 1.2, 1.6, 2.0 µg calcium per well. Bring the volume to total 50 µl with dH2O.
2. **Sample Preparation:** Serum or urine samples can be used directly in this assay. Place 10 µl samples in wells in a 96-well plate. For other liquid samples, add 2 – 50 µl sample into individual well. Bring the total volume to total 50 µl with dH2O. Samples can be assayed without any prior treatment. Some MRI contrast agents can cause transient interference in this assay.
3. **Additions:**
   - A) Add 90 µl of the Chromogenic Reagent to each well containing standards, samples or controls and mix gently.
   - B) Add 60 µl of the Calcium Assay Buffer to each well and mix gently.
4. Incubate the reaction for 5-10 minutes at room temperature. Protect from light.
5. Measure the OD at 575 nm. The chromophore is unstable and will fade slightly over time, so read the standard and samples within 30 minutes.
6. **Calculations:** Correct background by subtracting the value derived from the 0 Calcium control from all sample and standard readings (Note: The background reading may be significant and must be subtracted from sample readings). Plot standard curve µg/well vs. O.D. 575 nm readings. Then apply the sample readings to the standard curve to get Calcium sample amount in the wells (Sa). The Calcium concentrations in the test samples:

   C = Sa/Sv (µg/µl or mg/ml)

   Where: Sa is the Calcium Sample Amount (in µg) from standard curve.

   Sv is the Sample Volume (µl) added into the sample well.

   Calcium molecular weight: 40.

   Calcium concentration in your sample can be expressed as mg/ml, mg/dL or mM (mmol/liter).

   1 mg/ml = 100 mg/dL; 1 mM = 4 mg/dL.

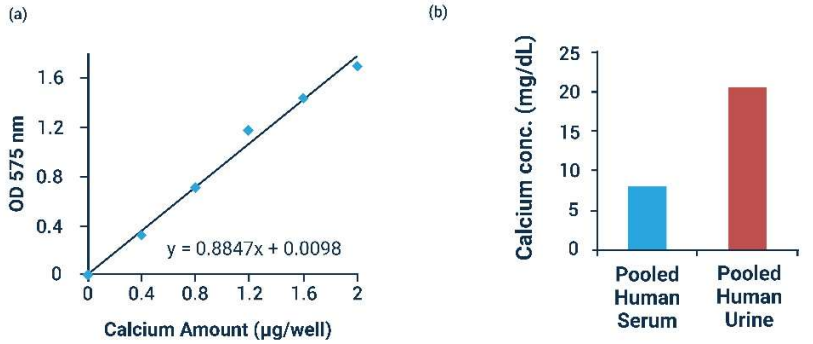

Figure: (a) Standard Curve. (b) Quantification of Calcium from pooled human serum (10 µl, 1:1 diluted) and pooled human urine (10 µl, 1:1 diluted). Assay was performed following the kit protocol.

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