BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Calcium Assay Kit (Cat #: A-132)

COMPONENTS: Calcium Assay Solution-I: 10 ml (for 200 wells), store at 4°C
Calcium Assay Solution-II: 20 ml, store at 4°C
4 mM $CaCl_2$: 1 ml, store at 4°C

PRODUCT DESCRIPTION: The Calcium Assay Kit is based on a modified o-cresolphthalein complexone method, where complexed calcium ions form violet color in an alkaline solution, enabling measurement of calcium ions by absorbance at 575 nm in 5 min. The assay is suitable for biological fluids such as serum/plasma, urine, saliva, and culture medium. Assay linearity is 0.2 – 5 mM. Kit components are stable for at least one year if stored and handled properly.

Calcium Standards:

Perform serial 1:1 dilution of the 4 mM calcium standard with dH_2O to obtain 2 mM, 1 mM, 0.5 mM, and 0.25 mM standards. Keep the calcium standard set refrigerated after use.

Sample preparation:

Serum/plasma in general can be used directly without dilution. If plasma is used, make sure EDTA or other chelators is not used. Samples should be centrifuged to remove insoluble material if necessary. Cell/tissue homogenates can be prepared by homogenization in 1% sodium dodecyl sulfate (SDS) using a mechanical homogenizer. Centrifuge cell/tissue homogenates in a microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and used for the assay. Samples containing calcium exceeding 5 mM should be properly diluted prior to assay.

Preparation of control solution and reaction solution:

Reaction solution is prepared fresh by mixing Calcium Assay Solution-I and Calcium Assay Solution-II in a 1:1 ratio. Control solution is prepared fresh by mixing dH_2O and Calcium Assay Solution-II in a 1:1 ratio. Each sample requires 100 µl reaction solution and 100 µl control solution. Calculate and prepare the amount of the solutions required prior to assay.

Assay

1. Use a plain (uncoated) 96-well plate for the assay. Add 10 µl dH_2O to a well and 10 µl of each calcium standard to a set of wells. Then add 10 µl of each sample to duplicate wells serving as control wells and reaction wells.
2. Add 100 µl reaction solution to the dH_2O well, each calcium standard well, and one set of sample reaction wells. Add 100 µl control solution to the other set of sample control wells. Gently agitate plate for 10 sec to mix contents.
3. Cover plate and keep plate on bench (room temperature) shielded from light for 5 min.
4. Read absorbance (O.D.) at 575 nm using a plate reader.
5. Subtract the dH_2O well reading from each calcium standard well reading. Generate a calcium standard plot using the subtracted standard readings (see graph below; x= calcium concentration in mM, y= $O.D._{575\ nm}$).
6. Subtract sample control well reading from sample reaction well reading for each sample. Use the subtracted sample readings for calculation of sample calcium concentration, which is derived from the trend line equation of the standard plot established at the same time.

ADDITIONAL INFORMATION:

- Samples should not contain the following chemicals: EDTA, sodium citrate, sodium fluoride, or oxalate.
- CAS-I contains hydrochloric acid and DMSO. Handle with caution and avoid skin contact.
- Please visit the product webpage or contact us for SDS information on hydrochloric acid, DMSO, and TEA.