ab102505 Calcium Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Calcium concentration in various samples. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit:
www.abcam.com/ab102505 (use abcam.cn/ab102505 for China, or abcam.co.jp/ab102505 for Japan)

Materials Supplied and Storage

Store kit at +4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components.
Aliquot components in working volumes before storing at the recommended temperature.
Reconstituted components are stable for 2 months.

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Calcium Assay Buffer	25 mL	+4°C	+4°C
Chromogenic Reagent	25 mL	+4°C	+4°C
Calcium Standard	100 µL	+4°C	+4°C

Materials Required, Not Supplied

- MilliQ water or other type of double distilled water (ddH_2O)
- Colorimetric microplate reader – equipped with filter for OD575 nm
- 96 well plate: clear plates for colorimetric assay
- Microcentrifuge
- Pipettes and pipette tips
- Orbital shaker
- Sonicator or Dounce homogenizer (if using cells or tissue)

1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

1.1 Calcium Assay Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at +4°C protected from light.

1.2 Chromogenic Reagent:
Ready to use as supplied. Equilibrate to room temperature before use. Aliquot reagent so that you have enough volume to perform the desired number of assays. Store aliquots at +4°C protected from light.

1.3 Calcium Standard:
Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays Store aliquots at +4°C protected from light.

2. Standard Preparation

Always prepare a fresh set of standards for every use.
Diluted standard solution is unstable and must be used within 4 hours.

2.1 Prepare a 5 mM Calcium standard by diluting 5 µL of the Calcium Standard in 495 µL of dH_2O.

2.2 Using 5 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	dH2O (µL)	Final volume standard in well (µL)	End Conc. Calcium in well
1	0	150	50	0 µg/well
2	6	144	50	0.4 µg/well
3	12	138	50	0.8 µg/well
4	18	132	50	1.2 µg/well
5	24	126	50	1.6 µg/well
6	30	120	50	2.0 µg/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

3. Sample Preparation

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

3.1 Tissue samples:
3.1.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10-100 mg).
3.1.2 Wash tissue in cold PBS.
3.1.3 **Resuspend tissue in 500 – 1,000 µL (or 4-6x the sample volume) of Calcium Assay Buffer and put on ice.**
3.1.4 Homogenize tissue with a sonicator. Alternative, use a Dounce homogenizer (10-50 passes) on ice.
3.1.5 Centrifuge samples for 2 -5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material. Collect supernatant and transfer to a clean tube.

3.2 Plasma, serum, urine samples and other fluids (growth medium):
Avoid the use of chelators such as EDTA when collecting serum. We recommend using heparin.
Biological fluids and other liquid samples can be tested directly by adding sample to the microplate wells.
However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

3.3 Cell Cultures:

3.3.1 Add 2x10^6 cells to 500 uL of Calcium Assay Buffer and sonicate on ice.
3.3.2 Centrifuge at top speed for 2-5 minutes at 4°C
3.3.3 Collect supernatant and transfer to a clean tube

4. Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use. It is recommended to assay all standards, controls and samples in duplicate.

4.1 Set up Reaction wells:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with dH_2O).

4.2 Add 90 µL of the Chromogenic Reagent to each well containing standards, samples and controls.

4.3 Add 60 µL of Calcium Assay Buffer into each well.

4.4 Mix and incubate at room temperature for 5-10 minutes protected from light.
Measure output on a microplate reader (OD575 nm).
The chromophore is unstable and will fade slightly over time, so read the standard and samples within 30 minutes.

5, Calculations:

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

5.1 Average the duplicate reading for each standard and sample.
5.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
5.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Calcium.
5.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Sa = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

5.6 Concentration of samples in the test samples is calculated as:

$$Calcium\ Concentration = \left(\frac{Sa}{Sv}\right) * D$$

Where:
Sa = Sample amount (in µg) from standard curve.
Sv = Sample volume (µL) added into the wells.
D = Sample dilution factor.

Calcium Molecular Weight is 40 g/mol.

FAQs:

Which anticoagulants do not interfere with this product?
Heparin is the only anticoagulant that does not interfere.

Will this assay work in presence of hemoglobin or in case of /hemolysis?
Assay will work with ≤ 750 mg/mL hemoglobin.

I want to measure Ca^{2+} in neutrophils. Is there a specific buffer for their preparation (lysis)?
You can use the calcium assay buffer provided with the kit. Use 2-5 x 10^6 cells, homogenize with the buffer, centrifuge and take the supernatant for the assay.

I want to use this product to measure the calcium contained in cell culture samples but have no access to a sonicator. What would you recommend for the sample preparation?
A Dounce homogenizer can be used. Alternatively freeze/thaw cycles with vortexing in the middle can also lyse the cells.

Interferences:

These chemicals or biological will cause interferences in this assay causing compromised results or complete failure.

- Heparin is the only anticoagulant that will work well with this kit. Other anticoagulants will interfere with color development.

Technical Support

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