

# **ab102505**

## **Calcium Detection**

### **Assay kit (Colorimetric)**

#### Instructions for Use

For the rapid, sensitive and accurate measurement of Calcium concentration in various samples.

[View kit datasheet: www.abcam.com/ab102505](http://www.abcam.com/ab102505)

(use [www.abcam.cn/ab102505](http://www.abcam.cn/ab102505) for China, or [www.abcam.co.jp/ab102505](http://www.abcam.co.jp/ab102505) for Japan)

This product is for research use only and is not intended for diagnostic use.

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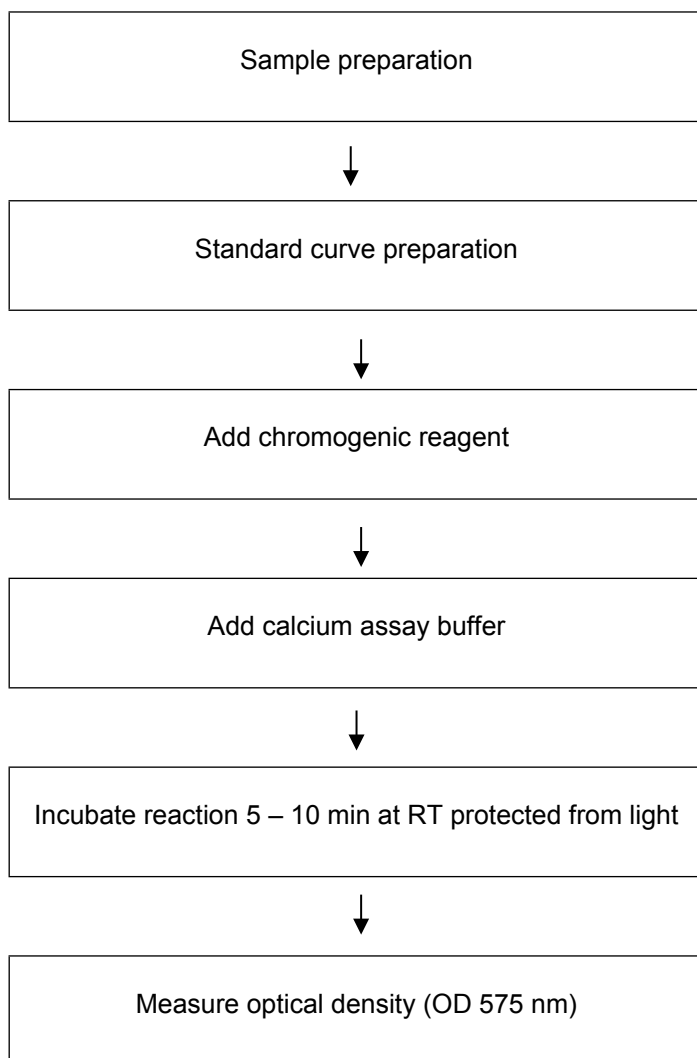
## 1. BACKGROUND

Calcium Detection Kit (Colorimetric) (ab102505) provides a simple assay to determine calcium concentration within the physiological range of 0.4 – 100 mg/dL (0.1 – 25 mM). A chromogenic complex is formed between calcium ions and 0-cresolphthalein and measured at OD = 575 nm. This product detects free calcium ions only.

Calcium is essential for all living organisms, where  $\text{Ca}^{2+}$  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.

## 2. ASSAY SUMMARY



### **3. PRECAUTIONS**

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

### **4. STORAGE AND STABILITY**

**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 in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

**5. MATERIALS SUPPLIED**

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Calcium Assay Buffer	15 mL	+4°C	+4°C
Chromogenic Reagent	25 mL	+4°C	+4°C
Calcium Standard (500 mM)	100 µL	+4°C	+4°C

**6. MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH<sub>2</sub>O)
- 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)

### 7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

### 8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

## 9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **Calcium Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at +4°C protected from light.

### 9.2 **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.

### 9.3 **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.

## 10. STANDARD PREPARATION

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

10.1 Prepare a 5 mM Calcium standard by diluting 5  $\mu\text{L}$  of the 500 mM Calcium Standard in 495  $\mu\text{L}$  of  $\text{dH}_2\text{O}$ .

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

Standard #	Volume of Standard ( $\mu\text{L}$ )	$\text{dH}_2\text{O}$ ( $\mu\text{L}$ )	Final volume standard in well ( $\mu\text{L}$ )	End Conc. Calcium in well
1	0	150	50	0 $\mu\text{g}/\text{well}$
2	6	144	50	0.4 $\mu\text{g}/\text{well}$
3	12	138	50	0.8 $\mu\text{g}/\text{well}$
4	18	132	50	1.2 $\mu\text{g}/\text{well}$
5	24	126	50	1.6 $\mu\text{g}/\text{well}$
6	30	120	50	2.0 $\mu\text{g}/\text{well}$

Each dilution has enough amount of standard to set up duplicate reading (2 x 50  $\mu\text{L}$ ).

## 11. SAMPLE PREPARATION

### **General Sample information:**

- 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.

### **11.1 Tissue samples:**

- 11.1.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10-100 mg).
- 11.1.2 Wash tissue in cold PBS.
- 11.1.3 Resuspend tissue in 500 – 1,000 µL (or 4-6x the sample volume) of Calcium Assay Buffer and put on ice. PBS + 0.1% NP-40 can also be used as an alternative to Calcium Assay Buffer
- 11.1.4 Homogenize tissue with a sonicator. Alternative, use a Dounce homogenizer (10-50 passes) on ice.
- 11.1.5 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.

### **11.2 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.*

### **12. ASSAY PROCEDURE and DETECTION**

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

#### **12.1 Set up Reaction wells:**

- Standard wells = 50  $\mu$ L standard dilutions.
- Sample wells = 1 – 50  $\mu$ L samples (adjust volume to 50  $\mu$ L/well with dH<sub>2</sub>O).

12.2 Add 90  $\mu$ L of the Chromogenic Reagent to each well containing standards, samples and controls.

12.3 Add 60  $\mu$ L of Calcium Assay Buffer into each well.

12.4 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.

## 13. 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).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Calcium.

13.4 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).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

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

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

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

Where:

Sa = Sample amount (in µg) from standard curve.

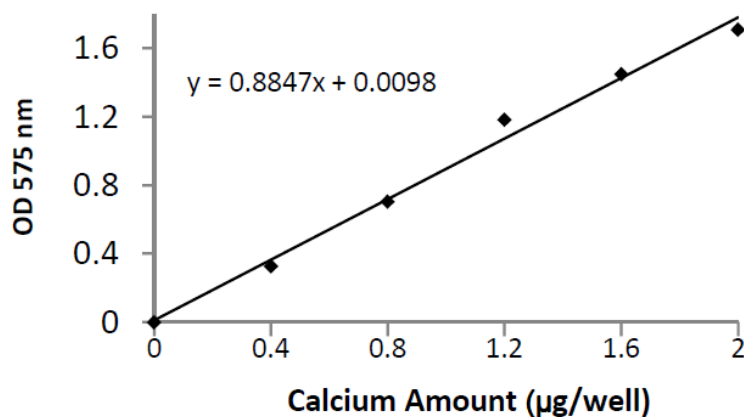
Sv = Sample volume (µL) added into the wells.

D = Sample dilution factor.

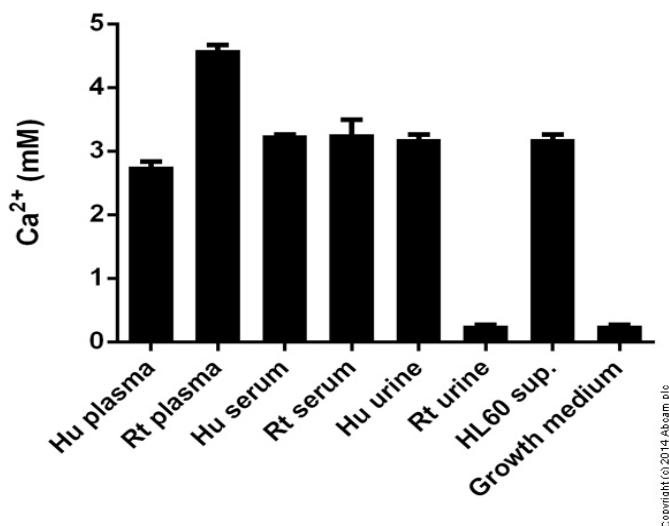
Calcium Molecular Weight is 40 g/mol.

## 14. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



**Figure 1:** Typical Calcium standard calibration curve using colorimetric reading.



**Figure 2:**  $\text{Ca}^{2+}$  measured in biological samples showing calcium concentration (millimolar).

### **15. QUICK ASSAY PROCEDURE**

**NOTE:** *This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Allow components to warm up to room temperature, get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50  $\mu$ L) and sample wells (50  $\mu$ L).
- Add 90  $\mu$ L of the Chromogenic Reagent to each well.
- Add 60  $\mu$ L of Calcium Assay Buffer into each well.
- Mix and incubate at RT for 5-10 minutes protected from light.
- Measure output (OD575 nm) on a microplate reader.

## 16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

## RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes ( $< 5 \mu\text{L}$ ) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

### **17. 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  $\leq 750$  mg/mL hemoglobin.

#### **I want to measure $\text{Ca}^{2+}$ in neutrophils. Is there a specific buffer for their preparation (lysis)?**

You can use the calcium assay buffer provided with the kit. Use  $2-5 \times 10^6$  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.

### **18.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.

### 19. NOTES



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