Calcium Assay Kit

Item No. 701220

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GENERAL INFORMATION

Materials Supplied

Item No.	Item	Quantity/Size
700551	Calcium Assay Buffer (10X)	1 vial/5 ml
700552	Calcium Standard	1 vial/2 ml
701221	Calcium Detector R1	1 vial/25 ml
701222	Calcium Detector R2	1 vial/25 ml
400014	96-Well Solid Plate (Colorimetric Assay)	2 plates
400012	96-Well Cover Sheet	2 covers

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling.Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 570-590 nm
2. Adjustable pipettes and/or repeating pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

About This Assay

Cayman's Calcium Assay provides a quick, reliable method for determining total calcium concentration in a variety of biological samples, as well as tissue homogenates and cell lysates. The assay utilizes an optimized variant of the well-established *o*-Cresolphthalein-calcium reaction in which a vivid purple complex is formed in the presence of calcium.

Calcium + *o*-Cresolphthalein Complexone ⟶ Calcium-Cresolphthalein Complexone Complex (A_{575})

In an alkaline environment, calcium reacts with *o*-Cresolphthalein Complexone to form a complex with a purple color that absorbs between 560 and 590 nm. The intensity of the color is directly proportional to the concentration of calcium in the sample.

PRE-ASSAY PREPARATION

Reagent Preparation

1. Calcium Assay Buffer (10X) - (Item No. 700551)

The vial contains 5 ml of concentrated Assay Buffer (1M Tris-HCl, pH 7.0). Dilute the entire contents of the vial with 45 ml of HPLC grade water. The diluted buffer (100 mM Tris-HCl, pH 7.0) is used to prepare the calcium standard curve and for sample dilution. When stored at 4°C, the diluted buffer is stable for at least six months.

2. Calcium Standard - (Item No. 700552)

The vial contains 2 ml of 20 mg/dl calcium standard in 0.1 M Tris, pH 7.0. The standard is ready to be used as supplied. When stored as supplied, the standard is stable for at least one year at 4°C.

3. Calcium Detector R1 - (Item No. 701221)

The vial contains 25 ml of calcium detector R1. It is ready to be used as supplied. When stored as supplied, the detector is stable for at least one year at 4°C.

4. Calcium Detector R2 - (Item No. 701222)

The vial contains 25 ml of calcium detector R2, an optimized solution of *o*-Cresolphthalein Complexone. It is ready to be used as supplied. When stored as supplied, the detector is stable for at least one year at 4°C.

5. Working Detector Reagent

To prepare the Working Detector Reagent, combine 10 ml of Calcium Detector R1 and 10 ml of Calcium Detector R2 and vortex lightly. The resulting solution is used in step 3 of the assay procedure (on page 13). This is sufficient reagent to assay one entire plate. Once mixed, the working detector reagent is stable for 60 minutes. Volumes can be adjusted depending on how many wells are to be used in the assay (*i.e.*, for 40 wells: 40 wells x 200 µl reagent per well = 8 ml of working reagent needed, or 4 ml of R1 + 4 ml of R2).

Sample Preparation

NOTE: All samples should have a pH value between 6 and 8. Values outside this range may affect the accuracy of the assay.

Plasma

Normal calcium levels in human plasma typically range from 8.9-10.4 mg/dl.

1. Collect blood using an anticoagulant such as heparin. (Do not use citrate, EDTA, or similar chelators which complex with calcium).
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80°C. The plasma sample will be stable for one month. Repeated freeze/thaw cycles should be avoided.
3. Dilute plasma 1:1 with diluted Assay Buffer prior to assaying.

Serum

Normal calcium levels in human serum typically range from 8.9-10.4 mg/dl.

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for one month. Repeated freeze/thaw cycles should be avoided.
4. Dilute serum 1:1 with diluted Assay Buffer prior to assaying.

Tissue Homogenate

1. Prior to dissection, rinse tissue with PBS (phosphate buffered saline solution, pH 7.4, containing 0.16 mg/ml heparin) to remove any extraneous red blood cells and clots.
2. Homogenize the tissue in 5-10 ml of phosphate buffered saline solution, pH 7.4, containing 0.16 mg/ml heparin per gram weight of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

Cell Lysate

1. Collect cells (~5 x 10^6) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, use a rubber policeman to collect cells.
2. Homogenize the cell pellet in 0.5-1 ml cold buffer (*i.e.*, 100 mM Tris, pH 7.5).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample should be stable for at least one month.

Urine

1. Collect urine in a suitable container.
2. Urine can be used directly in the assay, but may require dilution if calcium levels are particularly concentrated at the time of collection. If required, dilute the sample 1:2 with diluted Assay Buffer.
3. If not assaying on the same day, freeze the sample at -80°C. The sample should be stable for at least one month.
4. Urine is typically reported from a 24 hour collection period. Typical 24 hour urine collections yield 100-250 mg of calcium. However, if a single time point is desired, the calcium levels can be standardized against creatinine levels. The accepted reference interval for the calcium:creatinine ratio is less than 0.14. It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of calcium standards and samples to be measured in duplicate is given below in Figure 1. We suggest you record the contents of each well on the template sheet provided (see page 18).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H = Standards
S1-S40 = Sample wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 210 µl in all the wells.
- All reagents must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate (triplicate is recommended).
- Twenty-six samples can be assayed in triplicate or forty-two in duplicate.
- The assay is performed at room temperature.
- Monitor the absorbance at 570-590 nm.

Standard Preparation

Take eight clean glass test tubes or polystyrene tubes and label them A-H. Add the amount of Calcium Standard and diluted Assay Buffer to each tube as described in Table 1 below.

Tube	Calcium Standard (µl) (20 mg/dl)	Assay Buffer (µl)	Final Calcium Concentration (mg/dl)
A	0	100	0
B	2.5	97.5	0.5
C	5	95	1
D	10	90	2
E	20	80	4
F	30	70	6
G	40	60	8
H	50	50	10

Table 1. Preparation of standards

Performing the Assay

1. **Standard Wells** - add 10 µl of Calcium Standard (tubes A-H) per well in the designated wells on the plate (see Figure 1, on page 10).
2. **Sample Wells** - add 10 µl of sample to at least two wells per sample.
3. Add 200 µl of Working Detector Reagent (as prepared on page 7) to all wells being used.
4. Gently shake the plate for 20-30 seconds.
5. Incubate at room temperature for five minutes.
6. Read the absorbance at 570-590 nm. The resulting color is stable for at least 30 minutes.

ANALYSIS

Calculations

1. Calculate the average absorbance of each standard.
2. Subtract the average absorbance of standard A from itself and all other values (standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance of the standards (from step 2 above) as a function of the final concentration of calcium from Table 1. See Figure 2, on page 15, for a typical standard curve.
4. Calculate the calcium concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample.

$$\text{Calcium (mg/dl)} = \left[\frac{\text{Corrected sample absorbance - (y-intercept)}}{\text{Slope}}\right] \times \text{Sample dilution}$$

Performance Characteristics

Sensitivity:

This limit of detection for this assay is approximately 0.25 mg/dl.

Precision:

When a series of 80 calcium measurements were performed on the same day under the same experimental conditions, the intra-assay coefficient of variation was 3.0%. When a series of five samples were assayed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 5.96%.

Representative Standard Curve

The standard curve presented here is an example of the data typically provided with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use the one below to determine the values of your samples.

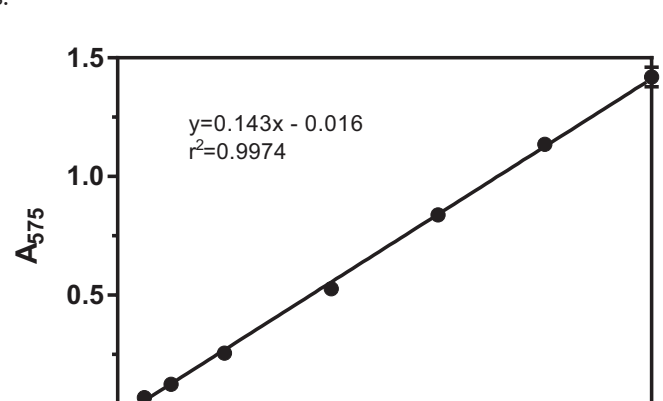


Figure 2. Typical standard curve

RESOURCES

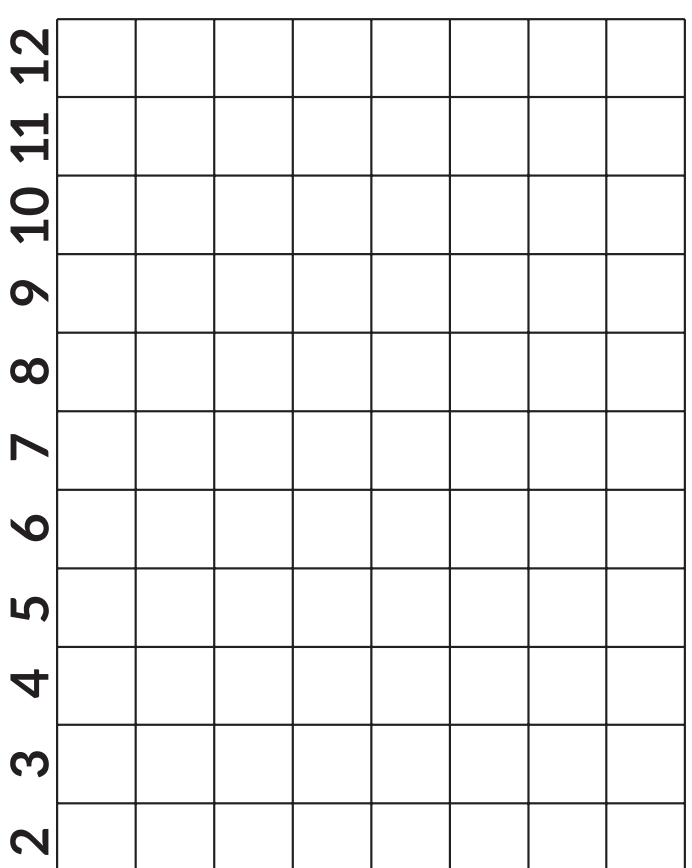
Interferences

The following reagents were tested for interference in the assay:

Reagent		Will Interfere (Yes or No)
Buffers	Tris	No
	MES	No
	Phosphate	No
	Borate	Yes
Detergents	Polysorbate 20 (1%)	No
	Triton X-100 (1%)	No
Chelators	Citrate	Yes
	EDTA (1 mM)	Yes
	EGTA (1 mM)	Yes
Protease Inhibitors/ Enzymes	PMSF (200 µM)	No
	Leupeptin (10 µg/ml)	No
	Antipain (10 µg/ml)	No
	Chymostatin (10 µg/ml)	No
	Trypsin (10 µg/ml)	No
Solvents	Methanol (5%)	No
	Dimethylsulfoxide (5%)	No
	Ethanol (5%)	No
Others	BSA (1%)	No
	Glutathione (1 mM)	No
	Glycerol (5%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Calcium was not detected in the sample	A. Sample pH is too low B. Sample was too dilute	A. Adjust sample pH to between 6 and 8 and re-assay B. Re-assay the sample using a lower dilution
Absorbance of samples fell above the standard curve	A. Sample pH is too high B. The concentration of calcium in the sample is too high	A. Adjust sample pH to between 6 and 8 and re-assay B. Dilute the sample to fall within the range of the standard curve
The calcium standard curve did not work	Either the calcium standards were not diluted properly or the standard has deteriorated	Set up the standards according to Table 1 on page 12 and re-assay



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.