Amplite™ Colorimetric Calcium Quantitation Kit **Blue Color**

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36361 (200 assays)	Keep at 4 °C Avoid exposure to light	Absorbance microplate readers

Introduction

Calcium is essential for all living organisms where movement of the calcium ion (Ca^{2+}) into and out of the cytoplasm functions as a signal for many cellular processes. Calcium is the fifth most abundant element by mass in the human body, where it is a common cellular ionic messenger with many functions, and also serves as a structural element in bone. Calcium plays an important role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. The serum level of calcium is closely regulated within a fairly limited range (9 to 10.5 mg/dL) in the human body. Both hypocalcemia and hypercalcemia are serious medical disorders. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels.

Amplite™ Calcium Quantitation Kit provides a simple method for detecting calcium in physiology solutions. This kit uses our Calcium Blue™ as the chromogenic calcium indicator. Its absorbance changes in response to calcium binding. The absorbance signal can be easily read by an absorbance microplate reader at 600 or 650 nm. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format within 5 minutes and easily adapted to automation without a separation step. With the Amplite™ Colorimetric Calcium Quantitation Kit, the calcium detection linear range is from 0.1 nmoles to 7.5 nmoles in 100 µL final test volume (2.5 to 150 µM calcium).

Kit Components

Components	**Amount**
Component A: Calcium Blue™	1 bottle (10 mL)
Component B: Dilution Buffer	1 bottle (20 mL)
Component C: 300 mM Calcium Standard	250 µL

Assay Protocol

Brief Summary

Prepare test samples (50 µL) or Calcium Standard solutions → Add Calcium Blue Reagent (50 µL) → Incubate at room temperature for 5-10 minutes→ Read the absorbance at 600 or 650 nm

Warm up all the components at room temperature before starting the experiment.

1. **Calcium standards and test sample preparations:**
   - 1.1 Take 10 µL of 300 mM Calcium Standard Solution (Component C) to 990 µL Dilution Buffer (Component B) to get 3 mM Calcium standard solution.
   - 1.2 Add 50 µL of 3 mM Calcium Standard solution (from Step 1.1) to 950 µL Dilution Buffer (Component B) to get 150 µM Calcium standard solution.
   - 1.3 Take 500 µL of 150 µM Calcium Standard Solution (from Step 1.2) to perform 1:2 serial dilutions to get 75, 37.5, 18.75, 9.375, 4.68, 2.34 and 0 µM serially diluted Ca^{2+}standards.
   - 1.4 Dilute the test sample to 5-100 µM range with Dilution Buffer (Component B).
   - 1.5 Add 50 µL of Calcium Standard, diluted samples and blank control into each well as shown in Table 1.

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Table 1. Layout of calcium standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
CS1	CS1	….	….	….	….						
CS2	CS2										
CS3	CS3										
CS4	CS4										
CS5	CS5										
CS6	CS6										
CS7	CS7										

Note: CS= Calcium Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well

Calcium Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Dilution Buffer (50 µL)	50 µL

**Note: Add the serially diluted calcium standards from 150 µM to 2.34 µM into wells from CS1 to CS7 in duplicate.*

2. Run calcium assay:

2.1 Add 50 µL of Calcium Blue™ (Component A) to each well of calcium standards, blank control, and test samples (see Step 1.5, Table 1 and 2) to make the total calcium assay volume to 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

2.2 Incubate the reaction for 5-10 minutes at room temperature, protected from light.

2.3 Measure the absorbance intensities at 600 nm or 650 nm**.**

3. Calcium calibration curve:

Plot the assay data as shown below.

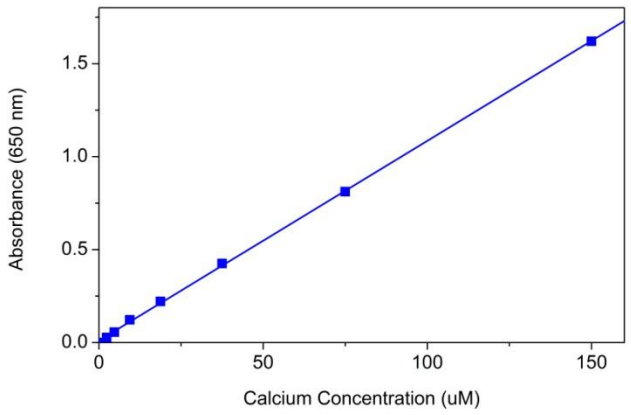

Figure 1. Calcium dose response was measured on a 96-well black plate with the Amplite™ Colorimetric Calcium Quantitation Kit. As low as ~ 2.5µM Ca^{2+} was detected with 5 minutes incubation time (n=3).

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Appendix 1. Assay Protocol for Serum or Urine Samples

Brief Summary

Prepare urine or serum samples (10µL) → Add Calcium Blue™ Assay Reagent (200 µL) → Incubate at room temperature for 5 minutes→ Read the absorbance at 600 or 650 nm

1. Prepare calcium standards and test sample preparations:

1.1 Take 10 µL of 300 mM Calcium Standard solution (Component C) to 990 µL Dilution Buffer (Component B) to get 3mM Calcium Standard Solution.

1.2 Take 500 µL of 3mM Calcium Standard Solution to perform 1:2 serial dilutions to get 1.5, 0.75, 0.375, 0.1875, 0.094, 0.047 and 0 µM serially diluted Ca^{2+} standards.

1.3 Add 10 µL of calcium standard, serum or urine samples and blank control into each well as shown in Table 3.

Table 3. Layout of calcium standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
CS1	CS1	….	….	….	….						
CS2	CS2										
CS3	CS3										
CS4	CS4										
CS5	CS5										
CS6	CS6										
CS7	CS7										

Note: CS= Calcium Standards, BL=Blank Control, TS=Test Samples.

Table 4. Reagent composition for each well

Calcium Standard	**Blank Control**	**Serum or Urine Sample**
Serial dilutions* (10 µL)	Dilution Buffer (10 µL)	10 µL

**Note: Add the serially diluted calcium standards from 3 mM to 0.047 mM into wells from CS1 to CS7 in duplicate.*

2. Run calcium assay:

2.1 Add 200 µL of Calcium Blue™ (Component A) to each well of calcium standard, blank control, and test samples (see Step 1.3, Tables 3 and 4) to make the total calcium assay volume of 210 µL/well.
Note: For a 384-well plate, add 2.5 µL of sample and 50 µL of assay reaction mixture into each well.

2.2 Incubate the reactions for 5-10 minutes at room temperature (protected from light).

2.3 Measure the absorbance intensities at 600 nm or 650 nm**.**

3. **Calcium calibration curve:**
   Plot the assay data as shown below.

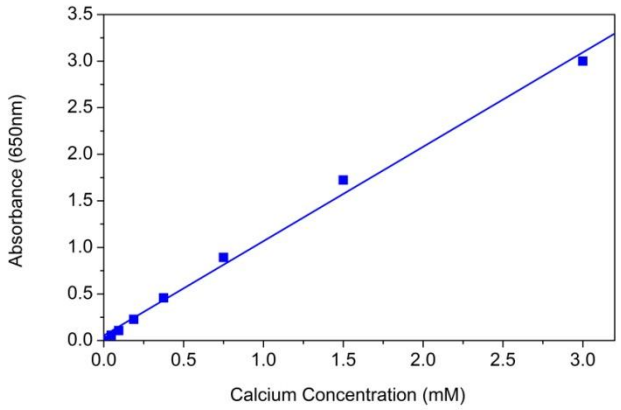

Figure 2. Calcium dose response was measured on a 96-well black plate with the Amplite™ Colorimetric Calcium Quantitation Kit. As low as 0.03 mM calcium was detected with 5 minutes incubation time (n=3).

References

1. Ilich JZ, Blanusa M, Orlic ZC, Orct T, Kostial K. (2009) Comparison of calcium, magnesium, sodium, potassium, zinc, and creatinine concentration in 24-h and spot urine samples in women. Clin Chem Lab Med, 47, 216.
2. Spano JC, Silva RG, Guedes DF, Sousa-Neto MD, Estrela C, Pecora JD. (2009) Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. J Endod, 35, 727.
3. Unkiewicz-Winiarczyk A, Bagniuk A, Gromysz-Kalkowska K, Szubartowska E. (2009) Calcium, magnesium, iron, zinc and copper concentration in the hair of tobacco smokers. Biol Trace Elem Res, 128, 152.
4. van Hulzen KJ, Sprong RC, van der Meer R, van Arendonk JA. (2009) Genetic and nongenetic variation in concentration of selenium, calcium, potassium, zinc, magnesium, and phosphorus in milk of Dutch Holstein-Friesian cows. J Dairy Sci, 92, 5754.
5. . Asai M, Takeuchi K, Uchida S, Urushida T, Katoh H, Satoh H, Yamada S, Hayashi H, Watanabe H. (2008) Misinterpretation of the effect of amlodipine on cytosolic calcium concentration with fura-2 fluorospectrometry. Naunyn Schmiedebergs Arch Pharmacol, 377, 423.
6. Gangidi RR, Metzger LE. (2006) Ionic calcium determination in skim milk with molecular probes and front-face fluorescence spectroscopy: simple linear regression. J Dairy Sci, 89, 4105
7. McNamara CJ, Perry TDt, Bearce K, Hernandez-Duque G, Mitchell R. (2005) Measurement of limestone biodeterioration using the Ca2+ binding fluorochrome Rhod- 5N. J Microbiol Methods, 61, 245.
8. David G, Talbot J, Barrett EF. (2003) Quantitative estimate of mitochondrial [Ca2+] in stimulated motor nerve terminals. Cell Calcium, 33, 197.