Technical Bulletin

Calcium Assay Kit

Catalogue Number MAK477

Product Description

Calcium is measured to monitor diseases of the bone or calcium regulation disorders. Increased calcium levels in serum are reported in hyperparathyroidism, metastatic bone lesions, and hypervitaminosis. Decreased levels of calcium are observed in hypoparathyroidism, nephrosis, rickets, steatorrhea, nephritis, and calcium-losing syndromes. Urinary calcium levels aid in understanding how the kidneys handle calcium in certain diseases of the parathyroid gland. Urinary calcium levels are also essential in the evaluation of kidney stones.

Simple, direct and automation-ready procedures for measuring calcium concentration in biological samples are useful in research and drug discovery. This Calcium Assay Kit is designed to measure calcium directly in biological samples without any pre-treatment. Phenolsulfonephthalein (phenol red) in the kit specifically forms a stable blue colored complex with free calcium. The intensity of the color, measured at 612 nm, is directly proportional to the calcium concentration in the sample. The optimized formulation minimizes any interference by substances such as magnesium, lipids, protein, and bilirubin.

The linear detection range of the kit is 0.08 – 20 mg/dL (20 µM – 5 mM). The kit is suitable for calcium determination in blood, urine, saliva, and other biological samples, as well as for studying the effects of drugs on calcium metabolism.

Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- Reagent A 50 mL
  Catalogue Number MAK477A
- Reagent B 50 mL
  Catalogue Number MAK477B
- Calcium Standard (20 mg/dL Ca^{2+}) 1 mL
  Catalogue Number MAK477C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

For Cuvette Method Only

- Cuvettes
- Spectrophotometer

For Whole Blood Samples Only

- EDTA disodium salt
  (Catalogue Number ED2SS or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Procedure

All samples and standards should be run in duplicate.

Assay can be performed at 37 °C or at room temperature. Prior to assay, bring the working reagents, 96-well plate, and spectrophotometer to the desired reaction temperature.

Briefly centrifuge small vials prior to opening.

Sample Preparation

EDTA and other Ca^{2+} chelators interfere with this assay. This assay cannot be applied to plasma samples obtained with EDTA.

No sample preparation is required for all sample types except whole blood. For whole blood, follow the Whole Blood Assay instructions.

Transfer 5 µL of each sample into a clear flat bottom 96-well plate. For cuvette procedure, transfer 15 µL of each sample into labelled test tubes.

Procedure Using 96-Well Plate

Standard Curve Preparation

1. Prepare standards in 1.5 mL microcentrifuge tubes according to Table 1. Diluted standards can be stored at 2-8 °C for future use.

Table 1.
Preparation of Calcium Standards

Well	20 mg/dL Standard	Purified Water	Calcium (mg/dL)
1	100 µL	-	20
2	80 µL	20 µL	16
3	60 µL	40 µL	12
4	40 µL	60 µL	8
5	30 µL	70 µL	6
6	20 µL	80 µL	4
7	10 µL	90 µL	2
8	-	100 µL	0

2. Mix well and transfer 5 µL of each Standard into separate wells of the plate.

Working Reagent

1. Mix enough reagents for the number of assays to be performed. For each Sample, Sample Blank (if used), and Standard well, 200 µL of Working Reagent is required. Prepare Working Reagent by mixing equal volumes of Reagent A and Reagent B, mix well.
2. Add 200 µL of Working Reagent to each Standard and Sample well. Tap lightly to mix.

Measurement

1. Incubate the plate for 3 minutes at the reaction temperature.
2. Read the optical density (OD) at 612 nm.

Procedure Using Cuvettes

1. Prepare Calcium Standards per Standard Curve Preparation section.
2. Set up test tubes for Standards and Samples. Transfer 15 µL of Standards and Samples to appropriately labelled tubes.
3. Prepare Working Reagent per Working Reagent section. 1000 µL is required per Sample or Standard tube.
4. Add 1000 µL of Working Reagent per tube and vortex to mix.
5. Incubate for 3 minutes at the reaction temperature.
6. Transfer to separate cuvettes and read the optical density (OD) at 612 nm.

Results

1. Calculate ΔOD by subtracting the OD reading of Standard #8 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against standard concentrations and determine the slope of the standard curve.
3. Calculate the calcium concentration of the Sample:

Calcium (mg/dL) =

$$\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$$

where:

OD_{Sample} = Optical density reading of Sample

OD_{Blank} = Optical density reading of Sample Blank or Standard #8 (Blank)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Conversions: 1 mg/dL Ca^{2+} equals 250 µM, 0.001%, or 10 ppm

Whole Blood Assay

To correct for interference in the sample matrix, two internal standard methods have been validated.

Protocol A is quicker whereas Protocol B is slightly more involved but requires less sample. Protocol B is recommended for customers that have a limited quantity of sample. Additionally, Protocol B requires less Reagent because each sample requires one well rather than three separate wells per sample.

Note: 20 mM EDTA is needed for whole blood sample preparation.

Protocol A - Three separate wells needed for each Sample

1. Whole Blood samples require an internal standard and need three separate reactions: 1) Sample plus Standard 2) Sample alone, and 3) Sample Blank.
2. For the internal standard prepare a 10 mg/dL Ca^{2+} Standard by mixing 125 µL of the 20 mg/dL Ca^{2+} Standard with 125 µL purified water.
3. Transfer 5 µL of whole blood sample to three separate wells.
4. Add 5 µL of the 10 mg/dL Ca^{2+} Standard to the well designated as Sample plus Standard.
5. Add 5 µL of purified water to the well designated as Sample.
6. Add 5 µL of 20 mM EDTA to the well designated as Sample Blank.
7. Add 200 µL of Working Reagent to each well (see Working Reagent section for preparation) and tap lightly to mix. Note: If any particulates or turbidity are seen, pipette up and down to dissolve.
8. Incubate for 3 minutes at room temperature and read optical density at 612 nm.

Protocol B - One well needed for each Sample

1. Prepare a 10 mg/dL Ca^{2+} Standard by mixing 125 µL of the 20 mg/dL Ca^{2+} Standard with 125 µL purified water.
2. Transfer 5 µL of whole blood sample to a well.
3. Add 200 µL of Working Reagent to the well (see Working Reagent section for preparation) and tap lightly to mix. Note: If any particulates are seen, pipette up and down to dissolve.
4. Incubate for 3 minutes at room temperature and obtain OD_{SAMPLE} value by reading the optical density at 612 nm.
5. Carefully transfer 5 µL of 10 mg/dL Standard to the Sample well from Step 2.
6. Tap plate to mix.
7. Incubate for 3 minutes at room temperature and obtain $OD_{STANDARD}$ value by reading the optical density at 612 nm.
8. Add 5 µL of 20 mM EDTA to the same Sample well from Step 2.
9. Tap plate to mix.
10. Incubate for 3 minutes at room temperature and obtain OD_{BLANK} value by reading the optical density at 612 nm.

Whole Blood Results

Calculate the whole blood sample concentration as follows:

Calcium (Ca^{2+}) (mg/dL) =

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Sample}} \; x \; 10 \; x \; DF$$

where:

OD_{Sample} = Optical density reading of Sample

OD_{Blank} = Optical density reading of Sample Blank

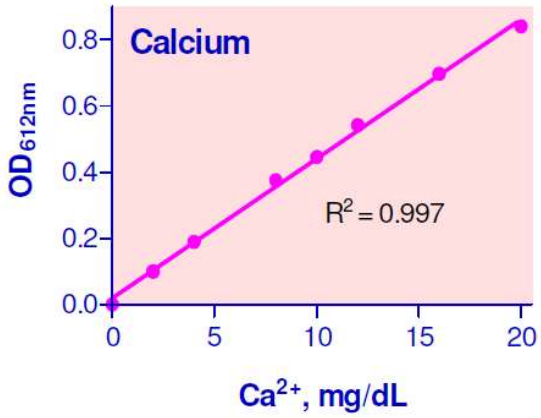
$OD_{Standard}$ = Optical density reading of Standard

10 = Concentration of the standard in mg/dL

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the calculated calcium concentration is greater than 10 mg/dL, dilute sample in purified water and repeat assay. Multiply result by the dilution factor (DF).

Typical Calcium Standard Curve



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