Calcium P FS*

Diagnostic reagent for quantitative in-vitro determination of calcium in serum, plasma or urine on photometric systems

Order Information

Cat. No.	Kit size						
1 1181 99 10 021	R1	4 x	20 mL	+	R2	1 x	20 mL
							+ 3 mL Standard
1 1181 99 10 026	R1	5 x	80 mL	+	R2	1 x	100 mL
1 1181 99 10 704	R1	8 x	50 mL	+	R2	8 x	12.5 mL
1 1181 99 10 917	R1	8 x	60 mL	+	R2	8 x	15 mL
1 1100 99 10 030		6 x	3 mL	Standard			

Summary [1,2]

Calcium plays an essential role in many cell functions: intracellular in muscle contraction and glycogen metabolism, extracellular in bone mineralization, in blood coagulation and in transmission of nerve impulses. Calcium in plasma exists in three forms: free, bound to proteins or bound to anions such as phosphate, citrate and bicarbonate in a complex reaction. Decreased total calcium levels can be associated with diseases of the bone apparatus (especially osteoporosis), kidney diseases (especially under dialysis), defective intestinal absorption and hypoparathyroidism. Increased total calcium can be measured in hyperparathyroidism, malignant diseases with metastases and sarcoidosis. Calcium measurements also help in monitoring of calcium supplementation mainly in the prevention of osteoporosis.

Method

Photometric determination with Phosphonazo III

Principle

At acidic pH calcium forms a purple-blue colored complex with phosphonazo III. In a second step calcium is bound to a chelating agent whereby the specific signal is eliminated. The resulting difference in absorbance is directly proportional to the calcium concentration in the sample. This guarantees a specific measurement of calcium.

Reagents

Components and Concentrations of the Reagents

R1:	Malonic acid buffer	pH 5.0	150 mmol/L
	Phosphonazo III		150 µmol/L
R2:	Malonic acid buffer		150 mmol/L
	Chelating agent		< 150 mmol/L
Standard:			10 mg/dL (2.5 mmol/L)

Storage Instructions and Reagent Stability

Reagents and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents! Protect the standard from light!

Warnings and Precautions

1. Reagent 1: Warning. H319 Causes serious eye irritation. H412 Harmful to aquatic life with long lasting effects. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention. P501 Dispose of contents/container to hazardous or special waste collection point.
2. As calcium is a ubiquitous ion, essential precaution must be taken against accidental contamination. Only use disposable materials.
3. Traces of chelating agent, such as EDTA can prevent the formation of the colored complex.
4. In very rare cases, samples of patients with gammopathy might give falsified results [5].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents and the standard are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or urine
Do not use EDTA plasma.

Stability [3]

in Serum/Plasma:	7 days	at	20 – 25°C
	3 weeks	at	4 – 8°C
	8 months	at	–20°C
in Urine:	2 days	at	20 – 25°C
	4 days	at	4 – 8°C
	3 weeks	at	–20°C

Discard contaminated specimens! Freeze only once!

Add 10 mL of concentrated HCl to 24 h urine and heat the specimen to dissolve calcium oxalate.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	660 nm
	700/800 nm bi-chromatic
Optical path	1 cm
Temperature	37°C
Measurement	against reagent blank

	Blank	Sample/ standard
Sample/standard	-	10 µL
Dist. water	10 µL	-
Reagent 1	1000 µL	1000 µL
Mix and incubate for 5 minutes. Read absorbance A1 than add:		
Reagent 2	250 µL	250 µL
Mix and read absorbance A2 within 1 minute.		

ΔA = (A2 – A1) Sample/standard

In case an absorbance of > 1.6 is observed after mixing of R1 and sample, dilute the sample 1 + 1 with NaCl solution (9 g/L), retest and multiply the result by 2.

Calculation

With standard or calibrator

$$\text{Calcium [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]}$$

Conversion factor

Calcium [mg/dL] x 0.2495 = Calcium [mmol/L]
Calcium/U [mg/24 h] x 0.025 = Calcium/U [mmol/24 h]

* fluid stable

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method has been standardized against the reference method Atomic Absorption Spectrometry (AAS). DiaSys TruLab N and P or TruLab Urine should be assayed for internal quality control. Each laboratory should establish corrective actions in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL
TruLab Urine Level 1	5 9170 99 10 062	20 x 5 mL
	5 9170 99 10 061	6 x 5 mL
TruLab Urine Level 2	5 9180 99 10 062	20 x 5 mL
	5 9180 99 10 061	6 x 5 mL

Performance characteristics

Measuring Range

The test has been developed to determine calcium concentrations within a measuring range from 0.2 – 25 mg/dL (0.05 – 6.24 mmol/L). When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, hemoglobin up to 1000 mg/dL, lipemia up to 2000 mg/dL triglycerides and magnesium up to 20 mg/dL. Strontium salts in medicine may lead to strongly increased calcium values. For further information on interfering substances refer to Young DS [4].

Sensitivity/Limit of Detection

The lower limit of detection is 0.2 mg/dL (0.05 mmol/L).

Precision

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	8.10	0.04	0.48
Sample 2	9.51	0.07	0.73
Sample 3	13.9	0.09	0.64

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	8.87	0.16	1.76
Sample 2	9.27	0.15	1.62
Sample 3	12.2	0.13	1.06

Method Comparison

A comparison of DiaSys Calcium P FS (y) with a commercially available test (x) using 84 serum samples gave following results:
y = 1.01 x - 0.142 mg/dL; r = 0.998

A comparison of DiaSys Calcium P FS (y) with a commercially available test (x) using 54 urine samples gave following results:
y = 1.01 x + 0.276 mg/dL; r = 1.00

Reference Range

Serum/Plasma [2]:
8.6 – 10.3 mg/dL (2.15 – 2.57 mmol/L)
Urine [1]:
Women < 250 mg/24 h (6.24 mmol/24 h)
Men < 300 mg/24 h (7.49 mmol/24 h)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 231–241.
2. Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1395–1406.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 20-1 and p. 50-1.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
5. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240–1243.

Manufacturer

IVD CE

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