



CALCIUM OCP

Colorimetric method for quantitative measurement of Calcium in serum, plasma and urine



ORDER INFORMATION

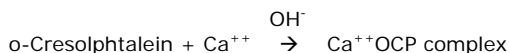
REF	Kit size
GA4325 00	5x50 + 5x50 ml
KL4325 00	4x20 + 4x20 ml

INDICATION

Conditions such as parathyroid disorders, neoplasms with or without bone metastasis, myelomas or other bone diseases can cause alterations in calcium levels.

METHOD PRINCIPLE

Calcium ions react, in alkaline medium, with O-Cresolphtalein forming a red-violet colour whose intensity is directly proportional to the calcium concentration in the sample.



Interference by magnesium is eliminated by addition of 8-hydroxyquinoline.

COMPOSITION

REAGENT A:

Ethanolamine buffer pH 10.7 1 mol/l

REAGENT B:

O-Cresolphtaleina	0.3 mmol/l
8-hydroxyquinoline	34.5 mmol/l

STANDARD:

	1x5 ml
Ca ⁺⁺	10 mg/dl
NaN ₃	0.95 g/l

Verified against NIST reference material.

PREPARATION OF REAGENTS

Bireagent procedure:

The reagents are liquids ready to use.

Monoreagent procedure:

Mix 1 part of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

Storage and stability

Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

Working reagent is stable for 1 day if stored at 20-25 °C and 3 days at 2-8 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, plasma (with heparin), 24h urine. Do not use anticoagulants such as EDTA, fluoride or oxalate.

After collection, separate the serum and plasma from the red blood cells as soon as possible to avoid the uptake of calcium by the erythrocytes.

Add 10 ml of concentrated HCl to 24h urine and heat the specimen to dissolve calcium oxalate, dilute 1:3 with distilled water.

Stability:

	Temperature		
	20-25 °C	2-8 °C	-20 °C
Serum, plasma	7 days	3 days	8 days
Urine	2 days	4 days	3 days

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known calcium concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	25, 30, 37 °C
Wavelength	575 nm (560-590 nm)
Optical path	1 cm
Reaction	End point (increase)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Reagent A	500 µl	500 µl	500 µl
Distilled H ₂ O	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix, incubate **1 minute**. Then add:

	Blank	Standard	Sample
Reagent B	500 µl	500 µl	500 µl

Mix and incubate for **5 minutes** at the desired temperature. Read absorbance value (A) of standard and samples against Blank.

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Working reagent	1000 µl	1000 µl	1000 µl
Distilled H ₂ O	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix and incubate for **5 minutes** at the desired temperature. Read absorbance value (A) of standard and samples against Blank.

Note:

- Reaction volumes may be proportionally changed.
- When plasma or serum concentration exceeds 20 mg/dl sample should be diluted 1:2 with NaCl solution (9 g/l) and the result multiplied by 2.

CALCULATION OF RESULTS

Siero, plasma:

$$\text{Calcium, mg/dl} = \frac{A \text{ sample}}{A \text{ standard}} \times 10$$

Urine (when 24h urine volume in known):

$$\text{Calcium, mg/24h} = \frac{A \text{ sample}}{A \text{ standard}} \times 300 \times l/24h$$

Conversion factor

$$\text{Calcium [mg/dl]} \times 0.2495 = \text{Calcium [mmol/l]}$$

$$\text{Calcium [mg/dl]} \times 0.4990 = \text{Calcium [mEq/l]}$$

REFERENCE VALUES

Serum-plasma: 8÷10 mg/dl (adults)

Urine 24h: 100÷300 mg/24h (varying according to diet)

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different calcium concentrations. The obtained results are reported in the following table:

Sample	Mean (mg/dl)	Within-run		Between-run	
		SD	%CV	SD	%CV
Serum 1	9.3	0.22	2.4	0.21	2.2
Serum 2	11.5	0.23	2.0	0.31	2.7
Serum 3	12.7	0.31	2.4	0.40	3.1

Linearity

The assay is linear up to 20 mg/dl.

Sensitivity

Test sensitivity, in terms of detection limit, is 0.2 mg/dl.

Correlation

A study based comparing this method with a commercial one gave the following results:

$$y = 0.985x + 0.15 \text{ mg/dl} \quad r = 0.99$$

Interferences

Bilirubin	> 40 mg/dl
Ascorbic acid	> 30 mg/dl
Magnesium	> 15 mg/dl
Hemoglobin	> 500 mg/dl
Triglycerides	> 2000 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended.

As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination.

Only use disposable materials.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

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6. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
7. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

 **Manufacturer:**

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