ROBONIK®

CE

prietest™ ...Clinical Chemistry Reagents

CALCIUM (OCPC)

In vitro diagnostic test kit, for professional use only

INTENDED USE : Quantitative in vitro determination of concentration of calcium in serum, plasma or urine on photometric systems.

ORDERING INFORMATION	**Pack Size**	**Cat No.**
	2 X 25 ml	CALOC 02 25
	2 X 50 ml	CALOC 02 50

CLINICAL SIGNIFICANCE : Calcium plays an essential role in many cell functions: intracellularly in muscle contraction and glycogen metabolism, extracellularly, in bone mineralization, in blood coagulation and in transmission of nerve impulses. Calcium is present in plasma in three forms: free, bound to proteins or complexed with anions as phosphate, citrate and bicarbonate. Decreased total calcium levels can be associated with diseases of the bone 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 test using O-Cresolphthalein Complexone (OCPC), End Point.

PRINCIPLE :
Ortho-Cresolphthalein Complexone reacts with calcium ions in alkaline medium forming a red-violet color. Interference by magnesium is eliminated by addition of 8-hydroxy-quinoline. The colour intensity is directly proportional to the serum total calcium concentration.

REAGENTS :

COMPONENTS AND CONCENTRATIONS:
N.B: Concentrations are those in the final test mixture.

R1 :

2- Amino 2-Methyl - 1 - Propanol	:	76 gm/l	
R2 :			
Hydrochloric Acid	:	0.060 mol/l	
8 - Hydroxy Quinoline	:	6.89 mmol/l	
O - Cresolphthalein Complexone	:	0.160 mmol/l	
Standard :	:	8.0 mg/dl	(2.00 mmol/L)

STORAGE INSTRUCTIONS AND REAGENT STABILITY :
The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 8°C and contamination is avoided. Don't allow to stand open, otherwise the pH decreases because of CO_2 absorption from the air. The standard is stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 8°C. Do not freeze the reagents!

WARNINGS AND PRECAUTIONS :
1. As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination. Only use disposable materials.
2. Take the necessary precautions for the use of laboratory reagents.

REAGENT PREPARATION :
Mix 1 volume of R1 with 1 volume of R2 to prepare working reagent. Example For preparation of working reagent of 10 ml, mix 5 ml of R1 with 5 ml of R2. Working Reagent solution is stable for 4 hrs at 20 to 25°C and 20 hrs at 2 to 8°C.

WASTE MANAGEMENT :
Please refer to local regulation requirements.

MATERIALS REQUIRED BUT NOT PROVIDED :
NaCl Solution 9 g/l, General laboratory equipment, Analyser / Photometer, Pipettes etc.

SPECIMEN :
Serum, heparinized plasma or urine. Do not use EDTA plasma.
Stability in serum / plasma:
7 days at 20 to 25°C, 3 weeks at 4 to 8°C, 8 months at – 20°C
Stability in urine:
2 days at 20 to 25°C, 4 days at 4 to 8°C, 3 weeks at – 20°C
Add 10 ml of concentrated HCl (6 N) to 24 h urine and heat the specimen to dissolve calcium oxalate. Discard contaminated specimens.

ASSAY PROCEDURE :
Application sheets for automated systems are available on request.

Wavelength	:	Hg 578 nm, 570 nm
Optical Path	:	1 cm
Temperature	:	37°C
Mode	:	End Point

Bring all the contents of the kit to Room Temperature prior to use.
Read absorbance of sample against reagent blank.
Label the test tube as blank, standard, sample, control and pipette into respective test tube the reagent, standard, sample, control sample as per the table given below :

	Blank	Standard	Sample / Control
Working Reagent	1000 µl	1000 µl	1000 µl
Deionised Water	10 µl	—	—
Standard	—	10 µl	—
Sample / Control	—	—	10 µl

Mix and read the absorbance (A) after 5 minutes incubation, but within 30 minutes.

CALCULATION :
With standard or calibrator.

$$\text{Conc. of unknown Sample} = \frac{\text{Concentration of Standard}}{\text{Abs. Standard} - \text{Abs. of Reagent Blank}} \times \text{Abs. of unknown Sample} - \text{Abs. of Reagent Blank}$$

CONVERSION FACTOR :
Calcium [mg/dl] X 0.2495 = Calcium [mmol/l]

CALIBRATION :
For the calibration of automated photometric systems use of the commercially available calibrator is recommended.

QUALITY CONTROL :
To ensure adequate quality, use of the commercially available control sera is recommended.

PERFORMANCE CHARACTERISTICS :

MEASURING RANGE :
The test has been developed to determine Calcium concentrations within a measuring range from 0.2 to 15 mg/dl (0.05 to 3.74 mmol/L). When values exceed higher limit of the range, such 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(1703.4 µmol/L), Bilirubin up to 40 mg/dl(684 µmol/L), Lipemia up to 1000 mg/dl (11.40 mmol/L) Triglycerides, Hemoglobin up to 0.5 g/dl (5 g/L) and Magnesium up to 15 mg/dl (6.17 mmol/L). A list of drugs and other interfering substances with Calcium determination has been reported by Young et al.

SENSITIVITY / LIMIT OF DETECTION :
The lower limit of detection is 0.2 mg/dl (0.05 mmol/L).

PRECISION :

Intra-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	13.57	0.11	0.81
Sample 2	12.7	0.10	0.79
Sample 3	14.3	0.11	0.77

Inter-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	11.6	0.22	1.90
Sample 2	12.7	0.17	1.34
Sample 3	12.4	0.24	1.93

METHOD COMPARISON :
A comparison between Robonik Prietest Calcium - OCPC (y) and a commercially available test (x) using 20 samples gave following results :

Linear Regression	:	y = 1.009x -0.504 mg/dl	
Correlation Coefficient	:	r = 0.8497	
REFERENCE RANGE :			
Serum / Plasma	:	8.6 to 10.3 mg/dl	(2.15 to 2.57 mmol/L)
Urine	:	Women < 250 mg/24 h	(<6.25 mmol/24h)
		Men < 300 mg/24 h	(<7.5 mmol/24h)

It is recommended that each laboratory should assign its own normal range.

LITERATURE :

1. Endres D.B., Rude R.K., *Mineral and bone metabolism*, Tietz Fundamentals of Clinical Chemistry, 5th Ed., Burtis, C.A. & Ashwood, E.R. W.B. Saunders eds. Philadelphia USA, (2001), 795.
2. Tietz N.W., Clinical guide to laboratory tests, 3 Ed., W.B. Saunders eds. Philadelphia USA, (1995), 100.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press, 1995.

INSTRUMENT APPLICATION ***prietest TOUCH***
Name : CALCIUM, Mod : END-P
Pri.: 578 , Sec.: 0
Temp: 37C , KF : 1.000
Vol : 500ul , Unit : mg/dl
Lag : 5 , Read : NA
Blk : Y, QC : Y, Norm : Y
Std : 1 , Concen :
Std.: 1 = 8.0
Normal HI = 10.3
Normal LO = 8.6
QCNH : *
QCNL : *
QCABH = *
QCABL = *
Rgnt. Linearity : 15
NOTE :
* Indicates user definable parameter.
NA Implies Not Applicable

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	CALCIUM
Reaction	End Point
Wavelength 1	578 nm
Temperature	37°C
Zero Setting	Reagent Blank
Standard Conc.	8
Units	mg/dl
Sample Volume	10 µl
Reagent Volume	1000 µl
Incubation Time	5 minutes
Reference Range	8.6 to 10.3
Reagent Linearity	15

ROBONIK®
An ISO 9001 : 2008 Certified Company
An ISO 13485 : 2012 Certified Company

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