Instructions for Use

For *in vitro* diagnostic use

CÁLCIO ARSENAZO III WS

Calcium Arsenazo III WS

Anvisa 80115310211

ORDER INFORMATION

Cat. No.	Kit size
3010250KWS	R: 1 x 250 mL
3010060MWS	R: 2 x 30 mL
3010060KWS	R: 2 x 30 mL
3010094.8RWS	R: 4 x 23,7 mL
3010040MKWS	R: 2 x 20 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Calcium in serum, plasma or urine on photometric systems.

SUMMARY [1,2]

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 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 calcium supplementation mainly in the prevention of osteoporosis.

METHOD

Photometric test using arsenazo III

PRINCIPLE

Calcium with arsenazo III at neutral pH yields a blue colored complex, whose intensity is proportional to the calcium concentration. Interference by magnesium is eliminated by adding 8-hydroxyquinoline-5-sulfonic acid.

REAGENTS

Components and Concentrations

Phosphate buffer	pH 7.5	50 mmol/L
8-Hydroxyquinoline-5-sulfonic acid		<10 mmol/L
Arsenazo III		<150 µmol/L

STORAGE AND STABILITY

Reagent is stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C, protected from light and if contamination is avoided. Do not freeze the reagents!

WARNINGS AND PRECAUTIONS

1. As calcium is an ubiquitous ion, essential precautions must be taken against accidental contamination. Only use disposable materials.
2. Traces of chelating agent, such as EDTA can prevent the formation of the colored complex.
3. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
4. In very rare cases, samples of patients with gammopathy might give falsified results [7].
5. Please refer to the safety data sheets (SDS) 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

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

The reagent is ready to use.

MATERIALS REQUIRED, BUT NOT PROVIDED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Human serum, heparin plasma or urine
Do not use EDTA plasma.

Stability [5]:

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

Add 10 mL of concentrated HCl to 24 h urine and heat the specimen to dissolve calcium oxalate.
Discard contaminated specimens. Only freeze once!

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

Wavelength	650 nm, Hg 623 nm, (630 - 670 nm)
Optical path	1 cm
Temperature	20 - 25 ºC / 37 ºC
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	10 µL
Distilled water	10 µL	-
Reagent	1000 µL	1000 µL
Mix, incubate for 5 min and read the absorbance against the reagent blank.		

CALCULATION

With calibrator

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

Conversion Factor

Calcium [mg/dL] x 0.2495 = Calcium [mmol/L]

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

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. Use Kovalent Topkon N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

PERFORMANCE CHARACTERISTICS

Measuring range

The assay is designed to determine Calcium concentrations within a measurement range of 0.04 – 20 mg/dL (0.01 – 5 mmol/L). When the values exceed this range, the 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 40 mg/dL, hemoglobin up to 500 mg/dL, lipemia up to 2000 mg/dL of

triglycerides and magnesium up to 15 mg/dL. Strontium salts in the medicine can lead to strongly increased Calcium values. For more information on interfering substances, see Young DS [6].

Sensitivity / Limit of Detection

The lowest detection limit is 0.04 mg/dL (0.01 mmol/L).

Precision

Intra-assay precision n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
Normal control	9.37	0.11	1.17
Pathological control	11.458	0.14	1.23

Inter-assay precision n = 9	Mean [mg/dl]	SD [mg/dl]	CV [%]
Normal control	9.59	0.16	1.65
Pathological control	11.61	0.12	1.06

Method comparison

Method comparison between Kovalent Cálcio Arsenazo III (y) and a commercial test (x) using 30 samples demonstrated the following results: $y = 1.0424 x - 0.125$; $R^2 = 0.9792$.

REFERENCE RANGE

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

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

LITERATURE

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998 p. 192-202.
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 1395-1457.
3. Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with arsenazo III. Anal Chim Acta 1971; 53:194-8.
4. Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. Anal Biochem 1981; 110:61-72.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 52 – 3.
6. 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.
7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	*In vitro* diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:

Kovalent do Brasil Ltda.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414 - Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310211	R: 2 x 50 mL
80115310211	R: 3 x 40 mL
80115310211	R: 5 x 40 mL

Costumer service: sac@kovalent.com.br - (21) 3907-2534 / 0800 015 1414

Expiration date and Lot no.: See label