BIOLABO
www.biolabo.fr
MANUFACTURER:
BIOLABO S.A.S,
Les Hautes Rives
02160, Maizy, France

CALCIUM Arsenazo III Method

Reagent for quantitative determination of calcium in human serum and plasma or urines.

REF 90004 **R1** 2 x 125 mL **R2** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision: www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

INTENDED USE

Reagent for quantitative determination of calcium in human serum and plasma or urines to assess calcium homeostasis. Laboratory professional use (manual or automated method).

GENERALITIES (1) (2)

Calcium fulfils a variety of roles in human physiology, not only as a structural factor in bones and teeth, but also in normal neuromuscular function and clotting of blood.
The level of serum calcium may be affected by intestinal malabsorption, by alterations in plasma proteins level, especially albumin, which should be measured concurrently with calcium.
Hypercalcemia is found in hyperparathyroidism, multiple myeloma, bone and parathyroidal neoplasms and in states with bones demineralization.
Hypocalcemia is encountered in hypoparathyroidism and in several cases of necrosis and acute pancreatitis.

PRINCIPLE (4)

At midly acidic pH, metallo-chromogen Arsenazo III combines with calcium to form a coloured complex which absorbance measured at 650 nm (640-660) is proportional to the amount of calcium in the specimen.

REAGENTS

R1 CALCIUM ARSENAZO III Reagent

Imidazol buffer pH 6.8 at 25°C	> 90	mmol/L
Arsenazo III	> 0.18	mmol/L
Surfactant	0.1	%
Preservative		

Danger Repro. 1B: H360 - May damage fertility or the unborn child

P201: Obtain special instructions before use,

P202: Do not handle until all safety precautions have been read and understood,

P280 Wear protective gloves/protective clothing/eye protection/face protection,

P308+P313: IF exposed or concerned: Get medical advice/attention,

P405: Store locked up,

P501: Dispose of contents/container in accordance with dangerous goods regulation. Classification due to: Imidazole < 1%

For more details, refer to Safety Data Sheet (MSDS)

R2 CALCIUM ARSENAZO III Standard

Calcium 10 mg/dL (2.5 mmol/L)

According to 1272/2008 regulation, this reagent is not classified as dangerous

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

I REAGENT PREPARATION

Ready for use.
On KENZA analyzers: fill a container with the reagent as follows:
- REF R000053_T on KENZA One
- REF R000071_T on KENZA 240 & 450.

For use on other analyzers: Use transparent dye-free containers.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 18-25°C, reagents are stable when stored and used as described in the insert:

Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- Transfer requested quantity, well recap vials and store at 18-25°C,
- Reagent (R1) is stable at least 3 months without contamination.

Discard any reagent if cloudy or if reagent blank measured at 650 nm is > 0.400 (manual procedure) or > 0.900 at 620 nm (automated analyzer).

SPECIMEN COLLECTION AND HANDLING (1) (2)

Serum or heparinised plasma:
Do not use citrate, oxalate or EDTA. Blood obtained on fasting patient with minimal venous occlusion and without exercise or after restoring circulation at least for 1 minute.
24 h Urines:
Acidify after collection with 20 to 30 mL HCl 6 N to dissolve calcium salts.
Dilute (1 + 2) with distilled water before performing the test.
Total calcium is stable in serum for:
- at least 7 days at 2-8°C.
- 6 months at -20°C.

Long-term freezing may lead to associated evaporation, lyophilisation or co precipitation with fibrin (i.e. heparinised plasma) or lipids.

LIMITS (3)

Handle with care specimens, calibrators and controls to avoid contamination by environmental calcium. Use disposable tubes and cuvettes and clean glassware with HCl 0.1N, well rinse with demineralised water.

Plastic and glass containers may adsorb calcium during storage, especially with diluted solution.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S

MATERIAL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

I QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO EXATROL-P Level II
- REF 95012 Urinary controls
- External quality control program

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFRENCE INTERVALS (2)

TOTAL CALCIUM in serum:

Population	mg/dL	mmol/L
Premature	6.2-11.0	[1.55-2.75]
0-10 days	7.6-10.4	[1.90-2.60]
10 days –24 months	9.0-11.0	[2.25-2.75]
24 months –12 years	8.8-10.8	[2.20-2.70]
12 years -18 years	8.4-10.2	[2.10-2.55]
18-60 years	8.6-10.0	[2.15-2.50]
60-90 years	8.8-10.2	[2.20-2.55]
> 90 years	8.2-9.6	[2.05-2.40]

TOTAL CALCIUM in 24 h Urines: < 300 mg/24 h (< 7.5 mmol/24 h)

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

On Kenza 240TX, 37°C, 620 nm, serum specimens:

Linearity Range: between 6 and 20 mg/dL

Detection limit: approx. 0.01 mg/dL

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	6.09	9.43	12.36
S.D. mg/dL	0.05	0.05	0.10
C.V. %	0.8	0.5	0.8

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	6.09	9.64	12.05
S.D. mg/dL	0.09	0.15	0.22
C.V. %	1.5	1.5	1.8

Comparison studies with commercially available reagent:

Realized on human sera (n=53) between 4.76 and 13.8 mg/dL

y = 1.0084x -0.03672 R= 0.9955

Analytical Sensitivity: approx. 0.054 abs for 1 mg/dL

Interferences:

Turbidity	Positive interference from 0.043 OD
Total bilirubin	Positive interference from 238 µmol/L
Direct bilirubin	No interference up to 406 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1089 mg/dL
Hemoglobin	Positive interference from 157 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 2 months

Calibration Stability: 2 months

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

Performances and stability data on Kenza 450TX/ISE and Kenza One are available on request.

CALIBRATION (5)

- REF 95015 BIOLABO Multicalibrator traceable *to SRM 909*
- REF 90004 (vial R2) for urines and manual method only

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

PROCEDURE

Manual method

Pipette into 1 cm path length cuvette (ambient). Temperature should be held constant as the absorbance of the dye is temperature sensitive.	
Reagent	1000 µL
Standard, Controls, Specimen	20 µL
Mix well. Let stand for 1 minute at room temperature. Read absorbance at 650 nm (620-660) against reagent blank. The coloration is stable for 1 hour away from light	

1. Urines:
   - Dilute specimen 1+2 in demineralized water
   - Use standard of the kit to calibrate (do not dilute)
2. Performances with manual procedure should be validated by user.
3. Performances on urines and plasmas should be validated by user.
4. Kenza applications and other applications proposal are available on request.
5. Bichromatic or multi-wavelengths analysis or specimen blank to may be used to reduce positive or negative interferences.
6. Bichromatic analysis: the 2nd wavelength is 700 nm

CALCULATION

Serum or plasma:

$$\text{Result} = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \times \text{Standard concentration}$$

Urines:

Multiply the above result by dilution factor 3

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1395-1406, p.1435-1439.*

(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 202-207*

(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-115 à 3-125*

(4) *BAUER J. P., Affinity and stochiometry of calcium binding Arsenazo III, Anal. Biol. Chem.(1981), 110, p.61-72*

(5) *SRM: Standard Reference Material ®*

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with