BIOLABO
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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
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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (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 hyperparathyroïdism, multiple myeloma, bone and parathyroïdal neoplasms and in states with bones demineralisation.
Hypocalcemia is encountered in hypoparathyroïdism 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

CALCIUM ARSENAZO III

R1 Reagent **Danger**

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

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: Imidazol < 1%

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

CALCIUM ARSENAZO III

R2 Standard

Calcium 10 mg/dL (2.5 mmol/L)
According to 1272/2008 regulation, this reagent is not classified as dangerous

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).
- 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.

REAGENT PREPARATION

Ready for use.

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 (vial R1) if cloudy or if reagent blank measured at 650 nm is > 0.400 (manual procedure) or > 0.900 (automated analyzer at 620 nm).

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. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with | H2O Demineralized water | Biological hazard

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. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (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 at 37°C on KENZA 240TX

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 on Spectrophotometer with commercially available reagent:

Realised on serum specimens (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
Haemoglobin	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

CALIBRATION (5)

- REF 95015 BIOLABO Multicalibrator traceable *to SRM 909c*
- Standard (vial R2)

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

PROCEDURE

Detailed Kenza 240TX procedure is available on request

Wavelength: 620 nm
Temperature: 37°C
Temperature should be held constant as the absorbance of the dye is temperature sensitive.

	Automated analyzer	Manual procedure
Reagent	250 µL	1000 µL
Standard, Controls, Specimen	5 µL	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

Notes:

1- For urines, use standard of the kit to calibrate (do not dilute) and control with REF 95012 (to be treated as patient's urines)

2- Performances and stability data's have been validated with serum on KENZA 240TX and KENZA 450TX

3-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user.

4- Applications proposal are available on request

5- Haemolysis, icterus, lipemia, paraproteins and magnesium: Perform bichromatic or multi-wavelengths analysis or specimen blank to reduce positive or negative interferences.

6- Bichromatic analysis: the 2nd wavelength is 700 nm

CALCULATION

Calculate the result as follows:

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 ®*