

INTRODUCTION

1. AutoZyme Calcium Arsenazo III is a reagent set for determination of total calcium concentration in serum based on the **colorimetric method using Arsenazo III**.
2. AutoZyme Calcium Arsenazo III is a **ready-to-use** reagent.
3. AutoZyme Calcium Arsenazo III estimates calcium in just **2 minutes at R.T. (25-30°C)**.
4. AutoZyme Calcium Arsenazo III is **linear** upto 15 mg%.
5. AutoZyme Calcium Arsenazo III can be used on any **Colorimeter, Spectrophotometer, Discrete semiautomated and Automated analyzer**. Programme can be designed for any specific analyzer upon request.
6. The **influence of lipids, haemolysis and bilirubin** is negligible.

PRINCIPLE

Calcium with Arsenazo III at neutral pH yields a blue coloured complex. The intensity of the colour formed is directly proportional to calcium concentration.

REAGENT STORAGE, STABILITY AND HANDLING

The kit should be stored at R.T. (25-30°C) and is stable till the expiry date indicated on the label.

Contamination of the reagent should be strictly avoided. Protect the reagent from direct light. Should the reagent develop turbidity discard the reagent.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Imidazol buffer; pH 7.0	100 mmol/l
• Arsenazo III	100 µmol/l
• 8-Hydroxyquinoline	5 mol/l
• Activators and stabilizers	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Plastic or siliconized container should be avoided as it may prolong clotting time. Serum is preferred but heparinised plasma (200 IU/ml blood) can also be used. EDTA, Citrate, Oxalate and Calcium salt of heparin interfere in the assay and should not be used as anticoagulant.

Calcium is stable in serum or plasma for 5 days when stored at 2 - 8°C and 20 days when stored at -10°C.

PROCEDURE

- Reaction type End-Point
- Reaction time 2 mins. at R.T. (25-30°C)
- Wavelength 650 nm. (620 - 650 nm.)
- Zero setting with Reagent Blank
- Blank absorbance limit < 0.800 Abs.
- Sample volume 0.02 ml (20 µl)
- Reagent volume 1.0 ml
- Standard concentration 10 mg%
- Linearity 15 mg/dl

Manual assay procedure

Perform the assay as given below :

1.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.02 ml	0.02 ml	—
Reagent	1.0 ml	1.0 ml	1.0 ml

Incubation

Mix and keep the assay mixture for 2 minutes at R.T. (25 - 30°C). Measure the absorbance of standard and sample against blank at 650 nm. (620 - 650 nm.). Final colour is stable for 1 hour if not exposed to direct light.

Calculation:

$$\text{Calcium in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 10$$

NOTE :

1. Avoid contamination of reagent into standard during its repeated use.
2. Glassware is the most common source of contamination in calcium assay. It is strongly recommended that glassware required for assay be rinsed with 0.1 N HCl followed by repeated rinsing with demineralized water.
3. The specimen and reagent volumes can be altered proportionally without affecting the final results.

EXPECTED VALUES

8.5 to 11.0 mg%

NOTE :

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range.

