CATACHEM

CALCIUM REAGENT KIT
C295-50

Contents	Product No.	Package
Calcium Reagent	**C295-50** C295-51	**4 x 250 mL** 1 x 250 mL

REAGENT PREPARATION

This reagent is packaged ready for use.
No preparation is required.

REAGENT STORAGE AND STABILITY

Store unopened reagent at 15-30°C.
When stored as directed, the reagent is stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: +1 203-262-0330

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone (203) 262-0330 ● catachem@catacheminc.com

CALCIUM REAGENT KIT
C295-50
MANUAL/AUTOMATED APPLICATION

Intended Use
For the quantitative determination of Calcium in serum using manual or automated applications.

Clinical Significance
Measurements of Calcium are primarily used for diagnosing hyperparathyroidism, steaorrhea, nephrosis, nephritis and pancreatitis, as well as for monitoring the causes and treatments. (1)

Method History
In 1971 Michaylova and Ilkova (2) reported the use of Arsenazo-III (o-(1, 8 dihydroxy-3, 6-disulfonaphthylene-2, 7 bisazo)-bis benzenearsonic acid) for the quantitative determination of micro amounts of Calcium in the presence of other metallic ions. The authors found that under acidic pH, magnesium does not interfere with the Ca^{++} reaction since at this pH Arsenazo-III does not form complexes with Mg^{++}. The strong specific affinity and its micromolar dissociation constant for the dye-calcium complex makes this metallochromic indicator Arsenazo-III a most suitable reagent for an accurate and rapid determination of Ca^{++} in serum samples.

Method Principle
The serum sample is mixed with the Calcium Reagent. The total free Calcium reacts with Arsenazo-III at pH 6.1 to form a purple-colored complex with maximum absorbance at 650nm. The increase in the purple-colored complex is directly proportional to the concentration of Calcium in the sample. The reaction scheme illustrates the reaction that occurs in the method.

$$2\ \text{Ars III} + Ca^{++} \xrightarrow{\text{pH 6.1}} Ca^{++}\ \text{Arsenazo Complex}$$

Reagent Content
The concentrations of the active ingredients in the reagents will be approximately as follows:

Calcium Arsenazo III Reagent
Each liter contains:

Buffer	
Arsenazo-III	0.15 mmol/L
Nonreactive ingredients and stabilizer	

Preparation Of Reagent
Catachem Calcium Arsenazo-III Color Reagent is packaged in ready-to-use form. No preparation is required.

Reagent Storage And Stability
Store Catachem Calcium Arsenazo-III Reagent at room temperature (15-30°C). When stored as directed, the reagent is stable until the expiration date stated on the label. The Catachem Calcium Arsenazo-III Reagent have been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection And Preparation
Test specimens should be fresh, clear non-hemolyzed sera. Old serum samples are not recommended since with age, protein will denature and precipitate together with calcium. Blood collected with anticoagulants such as oxalates and EDTA should be avoided since these anticoagulants will either precipitate or chelate Ca^{++} ions thus producing erroneous results.

Precautions
Avoid contact with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Quality Control
To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's Catacal (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Interfering Substances
Several substances have been reported to interfere with the Calcium method. (5) Care must be taken to use clean glassware and calcium-free distilled or deionized water. Anticoagulants such as oxalates and fluorides will depress calcium values. A summary of the interference of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (5)

Expected Values
The analytical measuring range of this assay as performed below is 7.5 mg/dL to 13 mg/dL (1.88 – 3.25 mmol/L). These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area where the laboratory is located.(1)

Directions For Use
Catachem's Calcium Arsenazo III method requires a single reagent. The reagent is packaged ready for use. No preparation is required. The Reagent is stable for twenty-four months from date of manufacture and for 60 days after opening if capped when not in use.

Procedure
***Important*:** Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation
Pipette	1.0 ml for reagent
Pipette	0.02 ml for sample

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● catachem@catacheminc.com

CALCIUM REAGENT KIT
C295-50
MANUAL/AUTOMATED APPLICATION

Materials Provided
Catachem Calcium Reagent

Analytical Parameters

Wavelength	650 nm or (600nm)
Pathlength	1 cm
Reaction Mode	endpoint
Reaction Time	2.0 minute
Reagent Volume	1.0 ml
Sample Volume	0.02
Total Volume	1:02 ml
Sample-to-reagent ratio	1:51

Assay Procedures
1. Pipette 1.0 ml of Calcium Reagent into each of three cuvettes marked “Calibrator”, “Sample” and “Blank”.
2. Pipette 0.02 ml of calibrator or sample into their respective cuvettes. Use 0.02 ml of distilled water for the Blank. Mix all cuvettes well.
3. Incubate all cuvettes for 2.0 minute at room temperature.
4. Set spectrophotometer wavelength at 650 nm (or 600 nm) and zero the instrument with the blank.
5. Read the “Calibrator” and “Sample” absorbencies.
6. Calculate the Calcium concentration (mg/dL) in the sample(s), as shown in calculations and results.

Calculations and Results

$$\text{Calcium (mg/dL)} = \frac{\text{Sample Absorbance (OD)}}{\text{Calibrator Absorbance (OD)}} \times \text{Calibrator (mg/dL)}$$

Example:

	Assay	OD
Sample		0.240
Calibrator		0.250
Calibrator	=	10.0 mg/dL

$$\frac{0.240}{} = 9.6 \text{ mg/dL}$$

$$\text{i.e. Calcium (mg/dL)} = \frac{\text{Sample OD}}{0.250} \times 10.0 \text{ mg/dL}$$

Method Performance Characteristics
***Sensitivity*:** The sensitivity of this method under normal circumstances is to 0.1 mg/dL (0.025 mmol/L)
***Linear Range*:** In this method there is no significant nonlinearity over the range of 2.0 - 20 mg/dL. (0.5 – 5 mmol/L)
***Precision*:** Within-run and day-to-day precision is summarized in the following table:

Precision Study

Calcium	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
2.6	0.00	0.00	0.22	8.40
12.3	0.00	0.00	0.18	1.40
20.4	0.13	0.64	0.36	1.80

Correlation
A comparison of this method using an automated analyzer and a reference method based upon the Arsenazo-III reaction resulted in the following regression statistics.

Range	=	7.5 - 15.3 mg/dL
N	=	106
Y	=	0.965x + 0.35
r	=	0.992
Sy.x	=	0.17

References
1. Fundamentals of Clinical Chemistry. Edited by Norbert Teitz. WB Saunders, Philadelphia (1976).
2. Michaylova V and Ilkova P. Anal Chim Acta 53, 194-198, 1971.
3. Bauer PJ. Anal Biochem 110, 61-72, 1981.
4. Ogan K. Simons ER. Anal Biochem 96, 70-76, 1979.
5. Young DA, Pestaner LC, Gibberman V. Effects of drugs on clinical laboratory tests. Clin Chem 21 (5) (1975).

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● catachem@catacheminc.com