

**Reagent kit for quantitative estimation of Calcium in Serum.**

**DIAGNOSTICS SIGNIFICATION:**

Calcium functions as an important factor in structure of bones and teeth, in neuromuscular activity and in clotting of blood. Elevated calcium values are associated with multiple myeloma, neoplasia of bone., hyperparathyroidism and conditions of rapid demineralization of bone. Lower calcium levels are associated with hypoparathyroidism, tetany and occasionally, with nephrosis or pancreatitis..

**PRINCIPLE:**

Ortho-Cresolphthalein Complexone reacts with Calcium at pH 10.0 to form a purple coloured complex. The colored complex formed is directly proportional to the calcium present when measured at 578 nm (565-580nm or with GREEN filter).



**SPECIMEN COLLECTION:**

Fresh, clear serum without hemolysis is necessary.

**PRESENTATION:**

Pack Size	2 X 25 ml	2 X 50 ml	2 X 100 ml
R1-Calcium (Dye Reagent)	1 X 25 ml	1 X 50 ml	1 X 100 ml
R2-Calcium (Buffer Reagent)	1 X 25 ml	1 X 50 ml	1 X 100 ml
Calcium Standard	1 X 01ml	1 X 01ml	1 X 02ml

**PRECAUTION:**

In case of glassware to be used in calcium assay they should be thoroughly decontaminated by soaking into 1N HCl overnight (8 to 10 hours) or alternatively, into 6N HCl for 4 to 8 hrs.

**PREPARATION OF WORKING REAGENT:**

R1-Calcium (Dye Reagent) & R2-Calcium (Buffer Reagent) are Ready-to-use.

**REAGENT STORAGE AND STABILITY:**

Calcium reagent and standard are stable at 2-8<sup>0</sup>C until the expiry date indicated on the label.

**ASSAY PARAMETER:**

Reaction : End point	Sample Volume : 10 µl
Wavelength : 578 nm	R1 + R2 Volume : 500 µl + 500 µl
Zero Setting : Reagent Blank	Standard Conc. : 10 mg/dl
Incub.Temp. : RT	Linearity : 20 mg/dl
Incub Time : 5 minutes	Unit : mg/dl

**PROCEDURE:**

Pipette into TT	Blank	Std.	Test
R1-Calcium (Dye Reagent)	500 µl	500 µl	500 µl
R2-Calcium (Buffer Reagent)	500 µl	500 µl	500 µl
Calcium Standard (10 mg/dl)	--	10 µl	--
Sample (Test)	--	--	10 µl

Mix and incubate at RT for 5 minutes. Read absorbance of Standard (S) and Test (T) after 5 minutes against reagent blank at 578 nm (565-580 nm or with GREEN filter).

**STABILITY OF FINAL REACTION MIXTURE:**

The color of the final reaction mixture is stable for 60 minutes when protected from Light and store at cold place.

**CALCULATION:**

Calcium concentration (mg/dl) = Abs T ÷ Abs S X 10

**NORMAL VALUES:**

Serum Calcium : 8.7 to 10.5 mg/dl.

**LINEARITY:**

The procedure is linear up to 20 mg/dl. If values exceed this limit, dilute the sample suitably with 0.9% saline and repeat the assay. Apply dilution factor to obtain the test results.

**REFERENCE:**

- Schwartzbach, G. Complexones and Their Analytical Applications, Analyst 80, 348-353, (1956).
- Zak, B., Epstein, E., Baginski, E.S., Review of Calcium Methodologies, Annals of Clinical and Laboratory Science 5, 195-215, (1975).

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