UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

CALCIUM

Product #: CA –F100 (100 Tests)

COLORIMETRIC DETERMINATION IN WINE & BEVERAGES

INTENDED USE

Calcium FLEX-REAGENT™ is intended for measuring Calcium ions in juice and wine.

KIT CONTENTS

	100T
Sample Blank Reagent	100 mL
Calcium R1, AMP Buffer	50 mL
Calcium R2, O-Cresophthalein	50 mL
Calcium Standard: 100 mg/L	5 mL

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 570 nm (540 - 600 nm) 0-2.0 absorbance range with a 1 cm lightpath, room temperature reaction.

SAMPLES

Centrifuge turbid sample and degas samples containing CO_2. Dilute wine samples containing ≥160 mG/L Calcium with DI water.

REAGENTS AND STORAGE

Kit contents are stable through the labeled expiration date when stored at 15-25°C. Reagents are liquid and ready to use. Close immediately after handling, protect from light.

ASSAY PREPARATION

Working Reagent: Prepare sufficient Working Reagent (1mL each of blank, standard and wine samples in your assay) by mixing equal parts Calcium R1 and Calcium R2 Reagents.
e.g. for 8 Samples: 5mL R1 + 5mL R2. This Working Reagent may be used for 1-day – stored at room temperature.
Sample Blank Reagent is ready to use.

TESTING PROCEDURE

Calcium results are corrected for Sample color by subtracting the ABS of each Sample (**Sample Blank** cuvette) from the respective reaction cuvette.
1. Pipet DI Water into the Reagent Blank cuvette, Calcium Standard into the Standard Cuvette, and each Sample into two (Reaction & Blank) cuvettes, as shown on the following table.
2. Pipet **Calcium Working Reagent** into the first set (Reagent Blank, Standard and Sample cuvettes) as shown;
3. Pipet **Sample Blank** Reagent into the Blank set of sample cuvettes, as shown.
4. Zero spectrophotometer with Reagent Blank Cuvette.
5. Mix and wait 5 minutes; read absorbance (ABS) of the, Reagent Blank, Standard, Samples & Sample Blanks.

	Reagent Blank	Standard	Sample	Sample Blank
D.I. Water	**25µL**			
Standard		**25µL**		
Samples			**25µL**	**25µL**
Working Reagent	**1 mL**			
Sample Blank Reagent				**1 mL**

Mix, wait 5 minutes

Zero Spec with Reagent Blank, read Absorbance (A 570nm)

CALCULATIONS

Calculate the delta ABS for each Standard and each Sample:

ΔABS_{Sample} =

$$\text{Calcium, mg/L} = \frac{Abs_{Sample} - ABS_{Sample\ Blank}}{ABS_{STD}}$$

LINEARITY: For samples with a calculated result > 160 mG/L, dilute and re-test sample; multiply result by the dilution factor.

SAFETY PRECAUTIONS

- Sample Blank and Reagent R1 may cause skin, eye, and mucous membrane irritation contact.
- The Iron Standard is corrosive.
- Handle reagents with caution, avoid swallowing and contact with skin, eyes and mucous membranes; wear suitable gloves and eye/face protection.
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Not environmentlally toxic, dispose of following local legal requirements.

APPENDIX

CHEMICAL PRINCIPLES

Calcium reacts with o-cresolphthalein complex in an alkaline solution to form an intense violet reaction product.
The intensity of the color is proportional to the concentration of Calcium in the wine sample.

TESTING PROCEDURE (Automation)

Contact Unitech Scientific for the ChemWell for Wine™ automated test procedures and technical support.

NOTES

1. Do not mix Reagents from different lots.
2. Specificity: this test is specific for Calcium. No interference was seen. This method is not affected by magnesium.
3. Use clean glassware, free from calcium traces. Otherwise wash the glassware with HCl diluted (2N) and rinse out with distilled water.

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