

UNITECH SCIENTIFIC

FLEX-REAGENT™

COPPER

Product #: **CU-20 (20 Tests)**

Di-Br-PAESA, Photometric

CU-50 (50 Tests)

INTENDED USE

Copper FLEX-REAGENT™ is specific for copper and intended for measuring wine copper concentrations.

KIT CONTENTS

	20-Tests	50-Tests
Reagent #1	18 mL	45 mL
Reagent #2	18 mL	45 mL
Reagent #3	4 mL	10 mL
Reagent #4	1 mL	2.5 mL
Copper Standards: 0.1mg/L	3 mL	3 mL
1mg/L	3 mL	3 mL

Also available from Unitech Scientific
High CU Control: 2mg/L, 3mL/vial

SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading Absorbance at 580nm [575 – 620] over a 0-2 A range, 1 cm light path

SAMPLES

Decolorize red wine by adding about 100mg of isinglass powder to 4 mL of red wine. Degas sparkling wines.

REAGENTS AND STORAGE

Reagents are liquid, ready to use, and stable through the labeled expiration date when stored at 2-8 °C and protected from direct light.

ASSAY PREPARATION

Prepare sufficient volumes of Sample Blank and Working Reagents using the ratio of components shown:

Sample Blank Reagent:

Mix **Reagent #1** and Reagent #3 prior to adding Reagent #4

Sample Blank Reagent	# of Cuvettes		
	10	30	50
Reagent #1	9mL	27	45
Reagent #3 (mix)	1mL	3mL	5mL
Reagent #4	0.25mL	0.75mL	1.25mL

Working Reagent

Mix **Reagent #2** and Reagent #3 prior to adding Reagent #4, as shown in the following table

Working Reagent	# of Cuvettes		
	10	30	50
Reagent #2	9mL	27	45
Reagent #3 (mix)	1mL	3mL	5mL
Reagent #4	0.25mL	0.75mL	1.25mL

Working Reagent & Sample Blank Reagent are stable 22 days at +2-8 °C.

TESTING PROCEDURE

1. Prepare and label two sets of cuvettes - Blank and Reaction. (Each set includes standards and samples.)
2. Pipet **0.1 mg/L & 1.0 mg/L Standards** and Samples into both sets of cuvettes, as shown on the following table.
3. Pipet Working Reagent into Reaction set of cuvettes; pipet **Sample Blank Reagent** into Blank set of cuvettes.
4. Zero spectrophotometer with DI Water.
5. Mix and wait 5 minutes; read absorbance (ABS) of all cuvettes.

ADD	Volume/Cuvette			
	Sample Blanking Cuvettes		Reaction Cuvettes	
	Standard	Samples	Standard	Samples
0.1 & 1.0mg/L Standard	300uL		300uL	
Sample		300uL		300uL
Sample Blank Rgt.	1000uL			
Working Reagent			1000uL	
Mix tubes, wait 5 minutes, and read absorbance (A 580nm)				

The method is linear to 2.0mg/L, if a calculated result is over-range, dilute sample and retest, multiply this test result by the dilution factor.

CALCULATIONS

Calculate the delta ABS for each Standard & Standard Blank and for each Sample and Sample Blank pair of cuvettes:

$$\Delta\text{ABS}_{\text{Sample}} = \text{Abs}_{\text{Sample}} - \text{ABS}_{\text{Sample Blank}}$$

$$\Delta\text{ABS}_{\text{STD}} = \text{ABS}_{\text{Standard}} - \text{ABS}_{\text{Standard Blank}}$$

$$\text{Copper (mg/L)} = \frac{0.9 \times (\Delta\text{ABS}_{\text{Sample}} - \Delta\text{ABS}_{\text{STD 1.0}})}{(\Delta\text{ABS}_{\text{STD 1.0}} - \Delta\text{ABS}_{\text{STD 0.1}})} + 1$$

QUALITY CONTROL

Monitor assay performance by testing a control in each assay. In order to monitor performance at a critical concentration, test a sample or prepare a custom dilution of the Standard provided (e.g. dilute 1.0 mg/L high Copper control 2-fold [0.2mL + 0.2mL DI Water] = **0.5mg/L**.) The recovered value should be within 25% of expected value. Factors that may affect the performance of this test include instrument function and pipetting accuracy (use calibrated micro-pipet).

APPENDIX

SIGNIFICANCE OF MEASUREMENTS

Copper is added to wine to address excess hydrogen sulfide content. In many regions, the legal limit of residual copper is 0.5 ppm. Copper may be removed from wine by a secondary treatment of yeast hulls.

PRINCIPLE OF THE METHOD

Under acidic conditions, copper reacts with Di-Fr-PAESA to form a colored complex, the intensity of the colored complex is proportional to the copper concentration in the sample.

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