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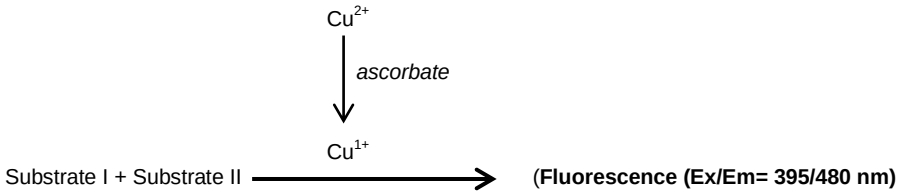
Copper Detection Assay Kit (Fluorometric)

12/18

(Catalog # K899-100; 100 assays; Store at - 20ºC)

I. Introduction:

Copper (Cu) is a d-block transition metal that occurs abundantly in nature. It is considered a micronutrient and is the third most abundant trace metal in the human body after iron and zinc (~70-100 mg). Copper supports several biological functions, primarily as a cofactor for enzymes like tyrosinase, amine oxidase and superoxide dismutase. The recommended dietary allowance for copper is ~900 µg/day. Excessive copper intake can lead to undesirable toxic effects and health conditions like liver disease. Since excessive copper intake can lead to copper toxicity, the U.S. Environmental Protection Agency (EPA) has set an upper limit for copper content in water supplies at 200 µM. Copper content in water supplies can increase due to industrial contamination or leaching of copper from supply pipes. Water supplies must be routinely tested to ensure that copper concentration is below this limit. BioVision's Copper Detection assay is a very simple one step plate-based assay that is highly selective for copper and free of interference from other contaminants in water supplies. The assay depends on a reaction catalyzed by Cu^{1+} ions that produces a highly fluorescent product. The rate of the reaction is proportional to the amount of copper present in water samples. The assay can detect as low as 0.1 nmol of copper and is linear up to 1 nmol.



II. Applications:

Measurement of copper concentration in environmental samples.

III. Sample Type:

- Environmental samples eg. tap water, bottled water, water from natural resources

IV. Kit Contents:

Components	K899-100	Cap Code	Part Number
Cu Assay Buffer	35 ml	NM	K899-100-1
Cu Substrate I	400 µl	Orange	K899-100-2
Cu Substrate II	1 ml	White	K899-100-3
Ascorbic acid	50 µl	Green	K899-100-4
Cu Standard 5 mM	100 µl	Yellow	K899-100-5

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay.

- **Copper Assay Buffer:** Warm to room temperature before use.
- **Copper Substrate I:** Thaw at room temperature before use. Aliquot and store the remaining stock at -20ºC.
- **Copper Substrate II:** Thaw at room temperature before use. Aliquot and store the remaining stock at -20ºC.
- **Ascorbic acid:** Thaw at room temperature before use. Aliquot and store the remaining stock at -20ºC
- **Copper Positive Control:** Thaw at room temperature before use. Aliquot and store the remaining stock at -20ºC

VII. Copper Assay Protocol:

1. **Sample preparation**: Environmental water sample may be used as is**.** For unknown samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range. Sample should be diluted using Copper assay buffer. Add up to 40 µl of the water sample into wells of the 96 well black plate. Adjust the volume of each well to 100 µl with Copper Assay Buffer.
2. **Standard Curve Generation:** Dilute the provided 5 mM Copper Standard 1:50 by adding 5 µl to 245 µl of copper assay buffer to obtain 100 µM copper solution. Add 0, 2, 4, 6, 8 and 10 µl of the 100 µM solution into a series of wells in a black 96-well plate to obtain 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol/ well. Adjust the volume of each well to 100 µl with Copper Assay Buffer.
3. **Reaction Mix:** Dilute an aliquot of the provided ascorbic acid solution at a dilution of 1:100 in Copper assay buffer. Prepare enough diluted ascorbic acid and mix enough reagents for the number of assays to be performed. Prepare 100 µl of reaction mix per well ***fresh prior to the experiments:***

	Reaction Mix
Copper Assay Buffer	78 µl
Copper Substrate I	4 µl
Copper Substrate II	10 µl
Ascorbic acid	8 µl

Add the reaction mix to all wells containing standards and samples of the 96-well black plate.

Notes:

a. Have the plate reader ready at Ex/Em = 395/480 nm on kinetic mode set to record fluorescence every 30 seconds.
b. Prepare reaction mix immediately before adding to wells.

4. **Measurement:** Immediately start recording fluorescence at 30 second intervals for 30 minutes. *Standard curve should also be read in kinetic mode.*
5. **Calculation:** Subtract the blank (0 nmol Copper) from copper standard (0.2-1 nmol) RFU values and sample RFU values. Obtain rate of reaction using the following equation:

Rate of reaction = $(RFU_{t2} – RFU_{t1}) / (t_2 – t_1)$; with t_2 and t_1 chosen in the reaction linear portion of the reaction

Obtain the standard curve by plotting Copper amount (nmol) on the x-axis and Rate of reaction for the respective copper amount on the Y axis (RFU/min). Apply background subtracted sample **Rate of reaction** to Standard Curve to get B nmol Copper in the sample well.

Copper concentration in sample: C = (B / V) X D (nmol/ml or µM)

Where **B** = amount of Copper in the sample well from Standard Curve (nmol)
V = volume of sample added into the well (ml)
D = dilution factor (in case sample was diluted before addition to reaction well).
t = time

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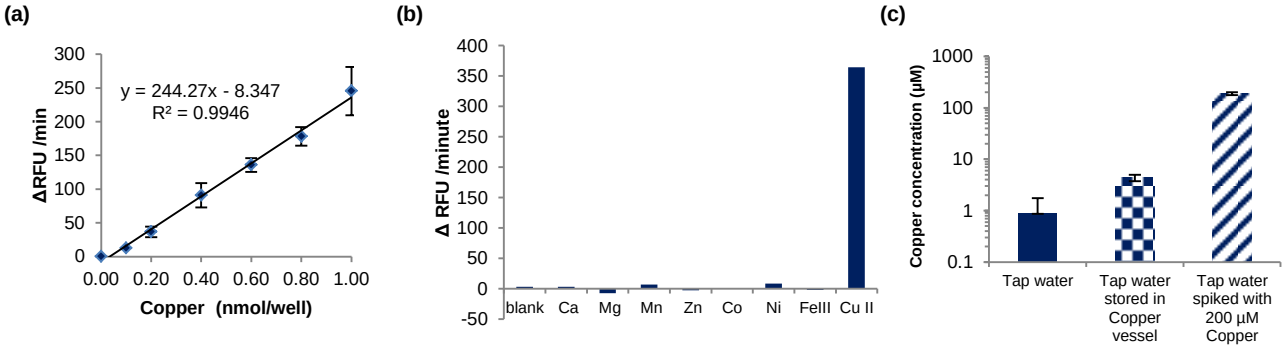

Figure 1: (**a**) Copper standard curve (**b**) Selectivity of the assay for Copper. Divalent and trivalent ions were tested at concentration of 20 mM vs copper at 2 mM **(c)** Copper concentration in different water samples. All experiments were done using kit protocol.

VIII. RELATED PRODUCTS:

Lithium Assay Kit (Colorimetric) K545
Sodium Assay Kit (Colorimetric) K391
Magnesium Colorimetric Assay Kit K385
Zinc Assay Kit (Fluorometric) (K428)
Calcium Assay Kit (Fluorometric) K409

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