# BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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## Copper Assay Kit (Cat #: A-120)

**COMPONENTS:** Copper Assay Solution: 20 ml (~150 wells), store at room temperature (RT)
Copper Control Solution: 20 ml, store at room temperature (RT)
Ascorbic Acid: 200 mg, store at room temperature (RT)
1 mM $CuSO_4$: 1 ml, store at room temperature (RT)

**PRODUCT DESCRIPTION:** The Copper Assay Kit is based on the reaction of bicinchoninic acid (BCA) with cupric ion and is suitable for analysis of cell/tissue samples and biological fluids (serum/plasma, saliva, urine, and culture medium). The reaction product has an optimal absorbance at 540 nm. The kit measures copper ions with a detection limit of 1-10 μM. Kit components are stable for several years if stored and handled properly.

### $CuSO_4$ Standard Set:

Dilute the 1 mM $CuSO_4$ solution 10x to 0.1 mM (100 μM), e.g., 0.1 ml 1 mM $CuSO_4$ + 0.9 ml $dH_2O$. Perform 1:1 serial dilution with $dH_2O$ to obtain 50 μM, 25 μM, and 12.5 μM $CuSO_4$.

### Sample preparation:

Serum/plasma: Mix 0.3 ml serum/plasma and 1.2 ml chloroform and vigorously vortex solution to ensure thorough mixing. Clarify solution by centrifugation at ~13,000 rpm for 10 min. Collect supernatant (top layer) for assay.

Cells/tissues: Freeze-thawed cells/tissues are mechanically homogenized in ice-cold $dH_2O$ followed by centrifugation at ~13,000 rpm for 5 min to remove insoluble materials. Collect supernatant for assay.

### Preparation of fresh reaction solution:

Prepare a fresh reaction solution by dissolving 20 mg Ascorbic Acid per ml Copper Assay Solution. Each well requires 125 μl reaction solution. Calculate the volume of reaction solution required for each assay. Keep reaction solution at room temperature and use immediately.

### Copper assay:

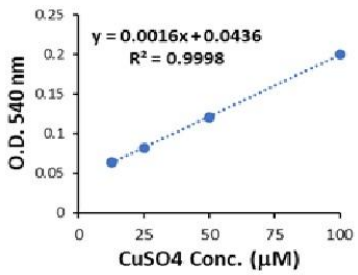
1. Use a plain (uncoated) 96-well plate for the assay. Add 125 μl $dH_2O$ to a well and 125 μl of each copper standard to a set of wells. Add 125 μl of each sample to duplicate wells serving as sample control and sample reaction wells.

2. Add 125 μl Copper Control Solution to one set of sample wells (control wells). Add 125 μl reaction solution to the $dH_2O$ well, each copper standard well, and the other set of sample wells (assay wells). Gently agitate plate to mix contents.

3. Cover and incubate plate in a 37°C incubator for 60 min. *Do not use $CO_2$ incubator.*

4. Read absorbance (O.D.) at 540 nm using a plate reader. Subtract the $dH_2O$ well reading from each copper standard well readings. Use the subtracted copper standard readings to generate a copper standard plot (see graph below; x= copper concentration in μM, y= $O.D._{540\ nm}$).

5. Subtract sample control well reading from sample reaction well reading for each sample. Use the subtracted sample readings for calculation of sample copper concentration as derived from the trend line equation of the standard plot established at the same time.




**ADDITIONAL INFORMATION:**

- Samples should not contain the following chemicals: EDTA, sodium citrate, sodium fluoride, or oxalate.
- Please visit the product webpage or contact us for SDS information on NaOH, BCA, potassium sodium tartrate, and cupric sulfate.