## Overview and Considerations:

**Note 1:** before starting check the "interfering substance chart" in the office. This assay does not work well in the presence of DTT (>0.1mM), the alternative assay is a Pierce 660.

**Note 2:** The linear range of this assay is ~0.2-50ug.

**Note 3:** the reagents for this assay are easily made, not kit required!

## Reagents:

**Reagent A:**

1 gm sodium bicinchoninate (BCA)

2 gm sodium carbonate

0.16 gm sodium tartrate,

0.4 gm NaOH

0.95 gm sodium bicarbonate

Brought to 100 ml with distilled water

Adjust the pH to 11.25 with 10 M NaOH

**Reagent B:**

0.4 gm cupric sulfate (5 x hydrated) in 10 ml distilled water.

**Working Solution:** Mix 50 volumes of reagent A with 1 volume of reagent B

Repeating pipet

Spectrophotomemter (WBSB 408) or 96 well plate reader (WBSB 516)

Eppendorf tubes/Cuvettes or 96 well plate

## Protocol

1. Prepare the working solution
2. Add 1ml of reagent to each tube (you will need 8 tubes for the standards, one tube for a buffer background, and one tube for each of your samples)
3. Add 0ul, 1ul, 2.5ul, 5ul, 7.5ul, 10ul, 12.5ul, or 15ul of 2mg/ml BSA to standard tubes, vortex well
4. Add 5ul each of samples to sample tubes, vortex after each addition.
5. Incubate tubes at 37oC for 30min
6. Read at 562nm
7. The curve should be linear out to 50ug.