A. Preparation of Diluted BSA Standard Protein

1. Dilute 5 mg/ml BSA Standard Protein into 0.5 mg/ml with 1xPBS buffer (137mM NaCl, 2.7mM KCl, 8mM Na2HPO4·7H2O, 1.5mM KH2PO4, pH 7.4).

2. Use Table 1 as a guide to prepare a set of diluted standards for working range (25-500 µg/ml)

Table 1. Preparation of Diluted BSA Standard Protein

Dilution Scheme for Standard Test Microplate Procedure (Working Range = 25-500 µg/ml)

Tube	Volume of Diluent (µl)	Volume of 0.5mg/ml BSA (µl)	Final BSA Concentration (µg/ml)
A	300	0	0
B	285	15	25
C	270	30	50
D	240	60	100
E	180	120	200
F	120	180	300
G	60	240	400
H	0	300	500

B. Preparation of the BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:
(8 standards + # unknowns) x (2 replicates) x (200 µl of WR per sample) = total volume WR required

2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of Reagent B (50:1, Reagent A:B).

C. Microplate Procedure (Sample to WR ratio = 1:8)

1. Pipette 20 µl of each standard or unknown sample replicate per well into a 96 wells microplate (working range = 25-500 µg/ml).

2. Add 200 µl of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.

3. Cover plate and incubate at 37°C for 30 minutes.

4. Cool plate to RT.

5. Measure the absorbance at 562 nm on a plate reader.