BCA Pretein Assay Procedure ThermoFisher cat # 23225 follow instruction on how to prepare working reagents, standards and control according to the manufacturer.

In short;

1. Pipette 25µL of each standard or unknown sample in triplicates into a 96 well plate (working range = 20-2000µg/mL) (e.g., Thermo Scientific Pierce 96-Well Plates, Product No. 15041).
- Note: if sample size is limited, 10µL of each unknown sample and standard can be used (sample to WR ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2000µg/mL.
2. Add 200µL 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, or RT for 2 hrs.
4. Cool plate to RT. Measure the absorbance at or near 562nm on a plate reader.
- Notes:
    - Wavelengths from 540-590nm have been used successfully with this method.
    - Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test tube Procedure. If higher 562nm measurements are desired, increased the incubation time to 2 hours.
    - Increasing the incubation time or ratio of sample volume to WR increases the net 562nm measurement for each well and lowers both the minimum detection level of the reagent and the working range of the assay. As long as all standard and unknowns are treated identically, such modifications may be useful.

WR = working reagent 50 parts reagent A and 1 part reagent B