

# BCA Assay

## Introduction

This protocol is adapted from ThermoFisher Scientific Pierce BCA Protein Assay Kit. Here the Microplate reader method (small volume 10-25  $\mu\text{L}$ ) is used to quantify the total protein concentration based on a colorimetric detection.

## Two steps:

Preparation of standards and Working Reagent (WR)

Preparation of the sample

## Materials

- Bovine Serum Albumin (BSA)
- Eppendorf tubes or vials
- Incubator at 37°C
- Sample for protein quantification
- MilliQ water (diluent)
- Plate reader
- 96-microtiter plates for assay

## Procedure

### Preparing of diluted BSA standards and WR

1. Dilute BSA using the following table.

Vial	Volume of Diluent [ $\mu\text{L}$ ]	Volume and Source of BSA [ $\mu\text{L}$ ]	Final BSA concentration [ $\mu\text{g/mL}$ ]
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 =Blank

2. Use the following formula to determine the total volume of WR required: (# standards + # unknowns)  $\times$  (# replicates)  $\times$  (volume of WR per sample) = total volume WR required Example: for the standard test-tube procedure with 3 unknowns and 2 replicates of each sample: (9 standards + 3 unknowns)  $\times$  (2 replicates)  $\times$  (2 mL) = 48 mL WR required Note: 2.0 mL of the WR is required for each sample in the test-tube

procedure, while only 200  $\mu$ L of WR reagent is required for each sample in the microplate procedure.

3. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). For the above example, combine 50 mL of Reagent A with 1 mL of Reagent B. Note: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

#### **Microplate procedure (sample to WR ratio = 1:8)**

1. Pipette 25  $\mu$ L of each standard or unknown sample replicate into a microplate well (working range = 20–2000  $\mu$ g/mL).  
Note: If sample size is limited, 10  $\mu$ L 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 is limited to 125–2000  $\mu$ g/mL.
2. Add 200  $\mu$ L 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. Measure the absorbance at or near 562 nm on a plate reader.  
Note: Wavelengths from 540–590 nm 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 562 nm measurements are desired, increase the incubation time to 2 hours. Increasing the incubation time or ratio of sample volume to WR increases the net 562 nm 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 standards and unknowns are treated identically, such modifications may be useful.
5. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in  $\mu$ g/mL. Use the standard curve to determine the protein concentration of each unknown sample.  
Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve provides more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

For further information, visit the ThermoFisher Scientific Pierce BCA Protein Assay Kit:

<https://assets.thermofisher.com/TFS->

[Assets/LSG/manuals/MAN0011430\\_Pierce\\_BCA\\_Protein\\_Asy\\_UG.pdf](Assets/LSG/manuals/MAN0011430_Pierce_BCA_Protein_Asy_UG.pdf)