# Pierce Rapid Gold BCA Protein Assay

## Introduction

The Pierce™ Rapid Gold BCA Protein Assay combines the protein-induced biuret reaction with the highly sensitive and selective colorimetric detection of the resulting cuprous cation (Cu1+) by a new, unique BCA chelator. An adaptation of the traditional BCA assay, the Rapid Gold BCA Protein Assay Kit has been optimized to develop in 5 minutes at room temperature. The Rapid Gold BCA Protein Assay produces an orange-gold colored reaction product, which is formed by the chelation of 2 molecules of the chelator with 1 cuprous ion. This water-soluble complex exhibits a strong absorbance at 480 nm that is very linear with increasing protein concentrations over a broad working range of 20-2000 µg/mL. In conjunction with the microvolume capability of a Thermo Scientific™ NanoDrop™ One spectrophotometer, the assay provides an accurate means of protein quantitation with minimal consumption of sample.

**Note:** All specifications and protocol instructions presented below are specific the pedestal mode for the NanoDrop One instrument. Please refer to the reagent manufacturer for additional guidance when utilizing the cuvette mode of the NanoDrop One.

## Dynamic Range

The Rapid Gold BCA Protein Assay has a linear range of 20-2000 µg/mL using a 1:20 sample to reagent ratio.

## Supplies

Equipment:

- NanoDrop One/One$^C$ Spectrophotometer
- 0.5 - 2 µL pipettor (and low retention tips), 10-100 µL pipettor, and a 100-1000 µL pipettor

Materials:

- Low lint laboratory wipes
- 0.5 mL microcentrifuge tubes or 0.2 mL mini-centrifuge strip tubes and caps or 96 well PCR plate (for standards and sample reactions)

Reagents:

- Pierce Rapid Gold BCA Protein Assay Kit (#A53225, A53227)
- BSA Standards (Pierce pre-diluted BSA standards #23208 or Pierce BSA standard ampules #23209) BGG Standards (Pierce pre-diluted BGG standards #23213 or Pierce BGG standard ampules #23212), or other protein standard
- PR-1 Reconditioning kit Part #CHEM-PR1-KIT

## Assay Recommendations

- Measure 2 µL sample aliquots
- Re-condition pedestals with PR-1 upon assay completion

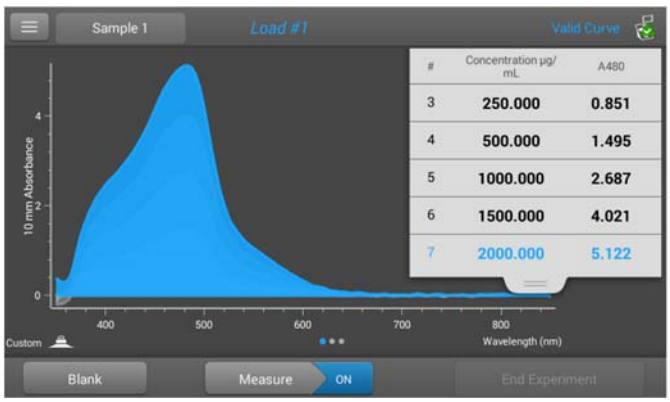
## Sample Preparation

1. Equilibrate all reagents, unknowns and protein standards to room temperature. Mix thoroughly but gently to avoid introducing micro bubbles.
2. Prepare enough fresh working reagent (WR) for all standards and samples to be measured using a 50:1 ratio of the kit reagents A:B.

**Note:** When Rapid Gold BCA Reagent B is first added to Rapid Gold BCA Reagent A, a pale blue precipitate may be observed, but, upon vortexing or mixing for < 5 seconds, the precipitate should dissolve to yield a clear, green solution. Use fresh working reagent each time.

3. Add the appropriate WR reagent volume to each microcentrifuge tube or PCR strip well.
   - **Rapid Gold BCA(1:10 sample to working reagent ratio):** Add 100 µL of working reagent to each standard and sample tube/well.
4. Add 10 µL of standards or samples to the appropriate tube. Mix well by gentle vortexing.
5. Incubate the standard and sample tubes at room temperature for 5 minutes.

**Note:** It is important to measure all standards and samples within 10 minutes or stop the reactions by adding 25 µL 1N HCl.




Typical absorbance spectrum for a Rapid Gold BCA protein assay sample measurement.

## Protocol

1. Select the **Custom** tab from the Home screen. Download Pierce Rapid Gold BCA method file for our website(https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/ultraviolet-visible-visible-spectrophotometry-uv-vis-vis/uv-vis-vis-instruments/nanodrop-microvolume-spectrophotometers/nanodrop-software-download.html) and place it on a USB drive. Then insert USB drive into the instrument and tap the **Custom** application button.
2. Select USB Drive and the select **Load Method**. Then select the Pierce Rapid Gold BCA method file and tap **Load** button**.** Then select **Run Method.**
3. Enter the values for each standard concentration in the table on the right. The software allows for the reference and up to 7 additional standards. Tap **Done**

   **Note**: The minimum requirement for standard curve generation is the measurement of two standards or the measurement of the zero reference and at least one standard. It is recommended that additional standards be included as necessary to cover the expected assay concentration range. Tap **Done**

   **Note:** If the instruments self-test begins, do not touch the instrument.
4. Prepare a blank solution using 10µL buffer+100 µL WR.
   - Pedestal Option: Pipette 2 µL of blank solution onto the bottom pedestal, lower the arm and tap **Blank**.
   - Cuvette Option (Model One$^{C}$ only): Insert the cuvette noting the direction of the light path indicated by the etched arrow. The optical beam (2 mm) is directed 8.5 mm above the bottom of the cuvette. Refer to the cuvette manufacturer for volume recommendations.

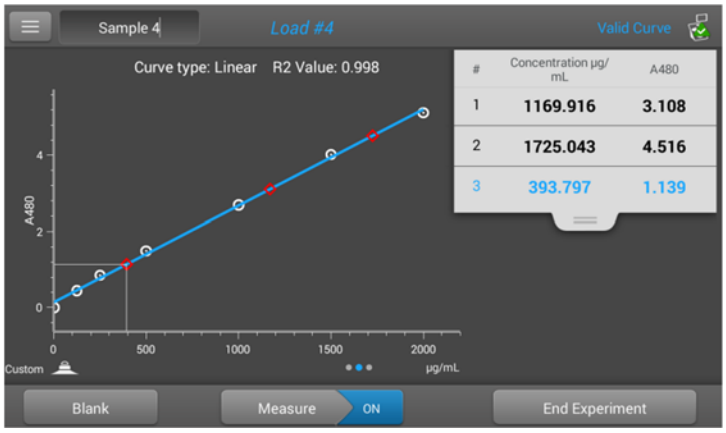
   **Note:** The arm must be down for all measurements, except those made with cuvettes. It is recommended that cuvettes be removed from the instrument prior to making a pedestal measurement to ensure that the pedestal arm can move to the proper starting position.
5. Follow the direction at the top of the screen to measure the standards. After each measurement, wipe the upper and lower pedestals using a dry laboratory wipe.
6. After all of the Standards have been measured, enter a sample ID and load a 2 µL aliquot of sample when using the pedestal. Tap **Measure**.
7. After completing all Standard and Samples measurements it is good practice to re-condition the pedestals using PR-1.
8. It is not necessary to blank the instrument between the standard and the unknown sample measurements.

   **Note:** A fresh aliquot of sample should be used for each measurement.

## Standard Curve Data

| BSA (µg/mL) | A480 |
|---|---|
| 125 | 0.446 |
| 250 | 0.851 |
| 500 | 1.495 |
| 1000 | 2.687 |
| 1500 | 4.021 |
| 2000 | 5.122 |

Typical absorbance values for an assay using 1:20 sample to reagent ratio assay using the Pierce Rapid Gold BCA reagent.




For additional information regarding the Rapid Gold BCA Protein assay and reagents:
www.thermoscientific.com/pierce