Protein Concentration Determination via Bradford Assay

Last updated: 2026-07-17

Reviewed by: Eleonora, Camille (Johans Protocol)

Materials:

- BSA Standard
- Bradford Reagent
- Buffer (diluted to 1x)

We have 100 µg/ml protein solution (BSA, bovine serum albumin) in 20 mM NaPHO + 500mM NaCl buffer. Create a calibration curve using BSA. Examine the linearity between 0 - 16 µg/ml with five points (make duplicates) see table below. 0 mg/ml is your reference point (blanking the spectrophotometer).

Spectrophotometer settings:

1. Find the program: Applications => single wavelength => Select following parameters:
   Absorbance 595 nm then press 'OK'.
2. You must blank (blue button) the spectrophotometer before you do your other measurements. Your 0 mg/ml with the Bradford reagent is your blank. You need to do this as a blank cuvette is used to calibrate the spectrophotometer by saving the baseline absorbance from your samples. It is analogous to "zeroing" a scale before weighing.
3. Make dilutions on the BSA original solution with the buffer used for protein expression (NaPHO + NaCl) directly in the cuvettes according to the table below and do not add the Bradford.
4. When you are ready to measure, add the Bradford solution, put a piece of parafilm over the cuvette and invert the cuvette so the liquids mix.
5. Each cuvette should incubate for 5 minutes (no more, no less) to allow the dye bond to reach equilibrium, then measure the absorbance at 595 m after 5 minutes and record, plot the average of the absorbance in two measurements.

Table for dilution of BSA:

BSA standard (100 µg/ml) **Units: µL**	**Buffer** **Units: µL**	**Bradford Reagent** **Units: µL**
0	800	200
20	780	200
40	760	200
80	720	200
120	680	200
180	640	200

Tips for making your calibration curve:

1. Label your axes properly (include units!)
2. Make sure you have the correct units!
3. -axis is the absorbance (what you measure) and the x-axis is the BSA concentration (given). Using the calibration cure that was created.