Nucleus Protocols

Pierce660 Assay

- ☐ Prepare a standard curve within the assay's working range (125 µg/mL to 2000 µg/mL). Remember to dilute the BSA stock in the same buffer used for your sample. The standards can be stored at –20 °C for future assays.
- ☐ Prepare a dilution series of your samples in the same buffer.
- ☐ Mix Pierce660 Reagent well by inverting the bottle before use.
- ☐ Array 150 µL of Pierce660 Reagent on a 96-well optical plate by reverse pipetting.
- ☐ Add 10 µL of each sample (BSA standard series and sample concentration series) column-wise (e.g., BSA standard in Column 12, Sample 1 series in column 1, ...) to the optical plate.
- ☐ Cover your plate with aluminum foil and mix on a plate shaker at medium speed for 1 min.
- ☐ Incubate your plate at 25 °C / 5 min. Samples should turn from brown to green.
- ☐ Using a plate reader, measure the absorbance of the samples at 660 nm.
- ☐ Analyze results.
    - ☐ Subtract the absorbance of blank samples (i.e., BSA standard = 0 mg/µL) from all other samples ("background subtracted absorbance").
    - ☐ Plot the standard curve by plotting the background subtracted absorbance vs. concentration for each BSA standard. Fit a line to your standard curve.
    - ☐ For each sample dilution series, choose a well with a background subtracted absorbance in the linear range of the standard curve.
    - ☐ Using the linear fit of your standard curve, calculate the concentration of the sample.