Correct determination of protein concentration in chromaffin cells

Background:

Due to high catecholamine contents in chromaffin cells most protein assays are not suitable for determining the total protein concentration in such cells. One example is given in Fig. 1 where two commercial protein assay reagents, Quant-iT Protein Assay Kit (Invitrogen) and BCA Protein Assay Kit (Pierce), are compared according to their reactivity with norepinephrine. Quant-iT reagent does not react with norepinephrine, whereas with the BCA reagent a concentration-dependent increase in absorbance is evident.

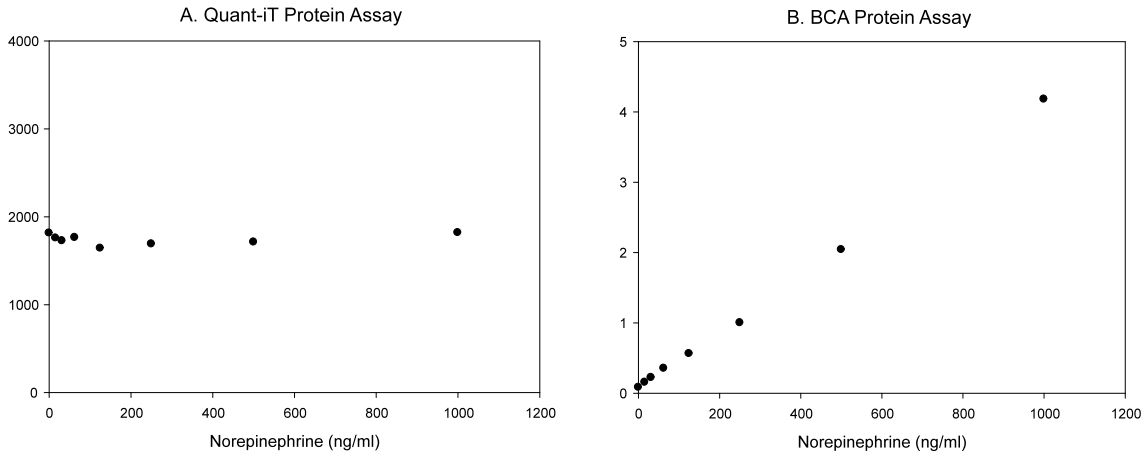

Fig. 1 – Norepinephrine reactivity with Quant-iT Protein Assay reagent (A) and BCA Protein Assay reagent (B)

Material and method:

In order to use Quant-iT Protein Assay Kit (Invitrogen), follow the manufacturer's instructions:

1. Equilibrate assay components to room temperature.

2. Make the working solution by diluting Quant-iT™ protein reagent 1:200 in Quant-iT™ protein buffer.

3. Load 200 µL of working solution in each microplate well. Diluted Quant-iT™ protein reagent is stable for at least 3 hours at room temperature, protected from light.

4. Add 10 µL of each of the Quant-iT™ protein standards to separate wells and mix well. Duplicates or triplicates of the standards are recommended.

5. Add 1–20 µL of each unknown protein sample to separate wells and mix well. Duplicates or triplicates of the unknown samples are recommended.

6. Measure fluorescence using microplate reader (excitation/emission maxima ~470/570 nm). The fluorescence signal is stable for 3 hours at room temperature.

7. Use a standard curve to determine protein amounts. For the BSA standards, plot amount vs. fluorescence, and fit a straight line to the data points.