

Protein Quantification Kit Protocol

1. Prepare serial dilution of protein standard in water. For example, a four-fold serial dilution of BSA from 200 µg/ml to 40 ng/mL is recommended. Ideally the protein standard curve will be prepared using the same protein species and in the same buffer as the sample protein of unknown concentration. This is not often possible, but whenever possible use the sample buffer for preparing the protein standard dilution series.
2. Take unknown sample and prepare a few dilutions in water. For example, measure the unknown sample neat, and diluted 1/10, 1/100 and 1/1000 in water.
3. Prepare assay diluents by mixing water, Part A and Part B 8:1:1. For example, mix 4 mL of water together with 500 µL of each of Part A and Part B.
4. Add an equal volume of kit diluents to each unknown sample. The recommended volumes are 50 µL of each as the kit is highly suited to microtitre plate array with a final volume of 100 µL. However, larger or smaller volumes if desired.
5. Once the unknown and assay diluent's are mixed, the samples are placed aside in the dark for 20 min or more prior to measurement. The signal is stable under these conditions for up to six hours. If longer period of storage are required it is recommended that the plates are sealed and stored at 4° C.
6. Once measurements have been recorded of the protein standards at known concentrations, plot fluorescence over protein quantity.

1 ASSAY VOLUMES

In microtitre plates assay volumes can range from 20 µL in 384-well plates to 100 or even 200 µL in 96-well plates. The kit works equally well for older methods of fluorescence quantification that involve large volumes (3 mL cuvettes), such as the Perkin Elmer Fluorometer.

2 SUITABLE INSTRUMENTATION

The kit is compatible with all industry-standard fluorescent imaging and recording systems that can excite with blue or green light and record red light emission. This includes the microtitre plate-based fluorometer such as the BMG FLUORostar. The laser-based imaging systems such as the, BIORAD FX and Molecular Dynamics Typhoon are also highly suited.

For full details and to order please contact FLUORotechnics at enquires@fluorotechnics.com