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Protein Concentration Determination using Qubit

In 1 collection

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In Development This protocol is published without a DOI.

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ABSTRACT

Procedure for quantification of protein concentration. This version is adapted for use with samples that have been extracted in protein extraction buffer as part of processing soybean/cowpea samples, but the general outline is similar for other samples. See the manual for further details: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_Protein_Assay_UG.pdf

PROTOCOL CITATION

Steven J Burgess 2020. Protein Concentration Determination using Qubit. **protocols.io** https://protocols.io/view/protein-concentration-determination-using-qubit-bqhkmt4w

COLLECTIONS

Immunoblot Analysis of Leaf Tissue

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PARENT PROTOCOLS

Part of collection

Immunoblot Analysis of Leaf Tissue

MATERIALS TEXT

- Qubit® protein assay kit (Life Technologies; Q33211)
- Qubit® assay tubes (Life Technologies; Q32856)
- Qubit® 4 Fluorometer (Life Technologies; Q33238)

ABSTRACT

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Create Working Solution for Analysis

Create a working solution of Qubit assay buffer by diluting the reagent 1:200 in the provided buffer.

1 The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit® working solution, and each sample tube requires anywhere from 180–199 µL. Prepare sufficient Qubit® working solution to accommodate all standards and samples.

Create Sample Dilution for Analysis 15m 6s

2 Dilute sample 1:50 (**196 µl** dH_2O + **4 µl** sample)

After taking into consideration the Qubit dilution factor (1:20; 10 µL sample + 190 µL qubit assay buffer) the sample being analyzed has been diluted 1:1000. This should give a value in the linear range for qubit (1.25-25 µg/mL). This should also reduce the impact of SDS on the quantification, which must be >0.2% in the 10 µL sample added (conc SDS in PEB is 2 %, so diluted 1:50 yields 0.04 %)

3 Add **190 µl** of Qubit working solution to a fresh Qubit assay tube, one for each sample to be analyzed (including the three protein standards)

4 Add **10 µl** of protein standard to the appropriate tube and mix by vortexing **00:00:03** s. 3s

5 Add **10 µl** of diluted sample to the appropriate tube and mix by vortexing **00:00:03** s. 3s

6 Allow samples to incubate at room temperature for **00:15:00** 15m

7 Measure protein sample concentration using the Qubit, following the instructions on the machine (i.e. start by measuring the standard curve).

Remember to adjust measured values according to the dilution factor applied. In this example, after taking into consideration the Qubit dilution factor (1:20; **10 µl** sample + **190 µl** Qubit assay buffer) the sample has been diluted 1:1000

Qubit™ 4 Fluorometer, with WiFi
Fluorometer
Invitrogen **Q33238**