Version 2

Dec 11, 2020

Protein Concentration Determination using Qubit 4 Fluorometer V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bqnbmvan

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ABSTRACT

Procedure for quantification of protein concentration using a Qubit 4 Fluorometer. The procedure follows the manufacturer's instructions, this version is adapted for use with samples that have been extracted in protein extraction buffer as part of processing soybean leaf tissue.

Consult the manual for further details:
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_Protein_Assay_UG.pdf

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PROTOCOL CITATION

Steven J Burgess 2020. Protein Concentration Determination using Qubit 4 Fluorometer. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqnbmvan
Version created by Steven Burgess

KEYWORDS

null, protein quantification, qubit, soybean, leaf tissue

IMAGE ATTRIBUTION

Image reproduced from ThermoFisher Scientific website https://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit/qubit-fluorometer.html

CREATED

Dec 11, 2020

LAST MODIFIED

Dec 11, 2020

PROTOCOL INTEGER ID

45475

MATERIALS TEXT

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

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BEFORE STARTING

This protocol assumes protein samples have been resuspended in 1x protein extraction buffer (62.5 mM Tris-HCl (pH 6.8), 2 % SDS (w/v); 10 % glycerol (v/v); 2.5% 2-mercaptoethanol (v/v)) and the dilution factors were determined in relation to processing of 3 size 7 Humboldt leaf disks resuspended in **450 µl** of buffer. Preliminary tests should be made when working with different samples or volumes

Create Working Solution for Analysis

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

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. Therefore prepare a 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).

The dilution is also important to reduce the impact of SDS and 2-mercaptoethanol on quantification. The concentration of SDS must be >0.2 %, and 2-mercaptoethanol > 20 mM in the 10 µL sample added to the assay. The concentration of SDS in PEB is 2 %, and 2-mercaptoethanol 335 mM, so diluted 1:50 yields [SDS] 0.04 % and [2-mercaptoethanol] 6.7 mM, which is in the acceptable range.

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

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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**

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