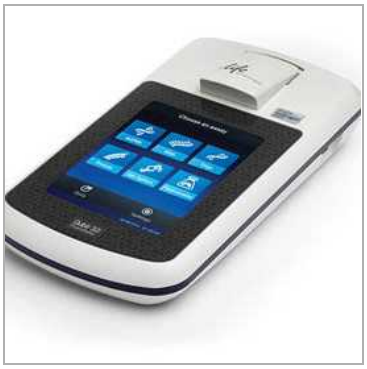

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We use this protocol and it's working

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Protein Concentration Determination using Qubit 4 Fluorometer

Forked from Protein Concentration Determination using Qubit 4 Fluorometer

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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

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

MATERIALS

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

BEFORE START INSTRUCTIONS

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.

> **Note**
>
> 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)

> **Note**
>
> 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

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).

Note

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

Equipment

Qubit™ 4 Fluorometer, with WiFi	NAME
Fluorometer	TYPE
Invitrogen	BRAND
Q33238	SKU
https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238	LINK