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Determining Protein Concentration of Cell-free Extract

In 1 collection

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ABSTRACT

Cell-free protein synthesis reactions require high-quality, active extracts to produce high yields of protein therapeutics, enzymes, antigens, vaccines, and other proteins. One way of assessing extract quality and lysis efficiency during cell-free extract preparation is to determine its overall protein concentration. This can be done using a Bradford assay.

Read more on cell-free protein synthesis here: https://www.swiftscalebio.com/blog/cell-free-protein-synthesis

ATTACHMENTS

dn6kbkfmx.pdf

DOI

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PROTOCOL CITATION

Weston Kightlinger 2021. Determining Protein Concentration of Cell-free Extract. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvgxn3xn

COLLECTIONS

Collection of protocols for cell-free protein synthesis

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PARENT PROTOCOLS

Part of collection

Collection of protocols for cell-free protein synthesis

MATERIALS TEXT

Materials:

1. 96 well plate with lid, Costar 3370
   Quick Start™ Bradford Protein Assay Kit 1 **BioRad**
2. **Sciences Catalog #5000201**
3. Plate reader
4. Cell-free extract

Determining Protein Concentration of Cell-free Extract

1

In 96 well plate with lid 3370 add **250 µl** of Bradford Reagent to each well.

2 Dilute BSA standards according to the following:

Microplate Standard Assay

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	20	2 mg/ml stock	0	2,000
2	30	2 mg/ml stock	10	1,500
3	20	2 mg/ml stock	20	1,000
4	20	Tube 2	20	750
5	20	Tube 3	20	500
6	20	Tube 5	20	250
7	20	Tube 6	20	125
8 (blank)	–	–	20	0

3 Make a serial dilution of extract (expected concentration of cell-free extract is ~ [M]**40 mg/ml**).

4 Measure all samples in triplicate [(# of samples+ 8 standards)*3=# of wells used].

5

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Add **5 µl** of sample/standard to each well containing Bradford Reagent.

6 Place plate on plate reader (must be able to read absorbance at 595 nm).

6.1 a. Turn on lamp and allow to warm up.

6.2 b. Set which wells to read in both protocol and procedure.

6.3 c. Plot standard curve using preferred software and use to calculate protein concentration from sample signal and dilution factor.

Expected Result: Expected concentration of protein in Cell-free extract is ~ **40 mg/ml** so make the following dilutions:

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