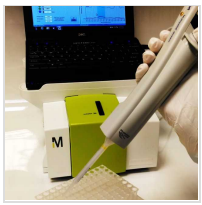

Aug 18, 2022

# Measuring protein concentration using the Merck Millipore Direct Detect Spectrometer

In 1 collection

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1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.8epv598m6g1b/v1

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ABSTRACT

This protocol details the procedure of measuring protein concentration using the Millipore Direct Detect spectrometer.

ATTACHMENTS

iiaebptmp.docx

DOI

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PROTOCOL CITATION

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COLLECTIONS

**Researcher led sample preparation for LC-MS using the BioMS research core facility**

KEYWORDS

Millipore Direct Detect, Measuring protein concentration, S-Trap buffer, protein estimation, spectrometer, quantitation, Bradford, BCA

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PARENT PROTOCOLS

Part of collection

Researcher led sample preparation for LC-MS using the BioMS research core facility

GUIDELINES

**Initial assumptions:**

- Allow approximately 20 minutes for measuring the protein concentration.
- You will prepare a pool of your sample for protein measurement.
- You have cell or tissue lysates in S-Trap lysis buffer (5% SDS with **50 millimolar (mM)** TEAB pH 7.5).
- Direct detect protein concentration measurement requires at least 6 uL of your sample.
- Protein lysates have been reduced and alkylated and clarified by centrifuging at **14000 x g** for **00:10:00** (see reduction and alkylation protocol).
- You will also need a blank sample to measure against. The blank is the buffer/solvent used to prepare the sample, but there is no protein in it. This should be 5% SDS with **50 millimolar (mM)** TEAB pH 7.5 containing **10 millimolar (mM)** DTT with **15 millimolar (mM)** IAM.
- If you wish to do more than a single pooled measurement, you may buy a box of direct detect cards from us using PPMS (https://corefacilities.manchester.ac.uk/?BioMS).
- A box costs £82.50 (price correct as of August 2022).



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MATERIALS TEXT

Direct Detect®
Spectrometer
**Merck Millipore** **C134681**

Direct Detect® Assay-free Cards
**Merck Millipore** **DDAC00010-GR**

BEFORE STARTING

**Initial preparation**

**Before you begin:**

**Identify the following equipment that you will use:**

- 10uL pipette and pipette tips
- Millipore Direct detect sample cards (DDAC00010-GR)
- Millipore Direct detect machine (C134681)

**Loading samples on card:**

1 To measure the protein concentration of your lysate, place a Direct Detect card on a clean, dry surface (spotting trays for cards are available).

The Direct detect is located in lab B2075.
There are spotting trays available on the shelf above the Direct Detect (DD).





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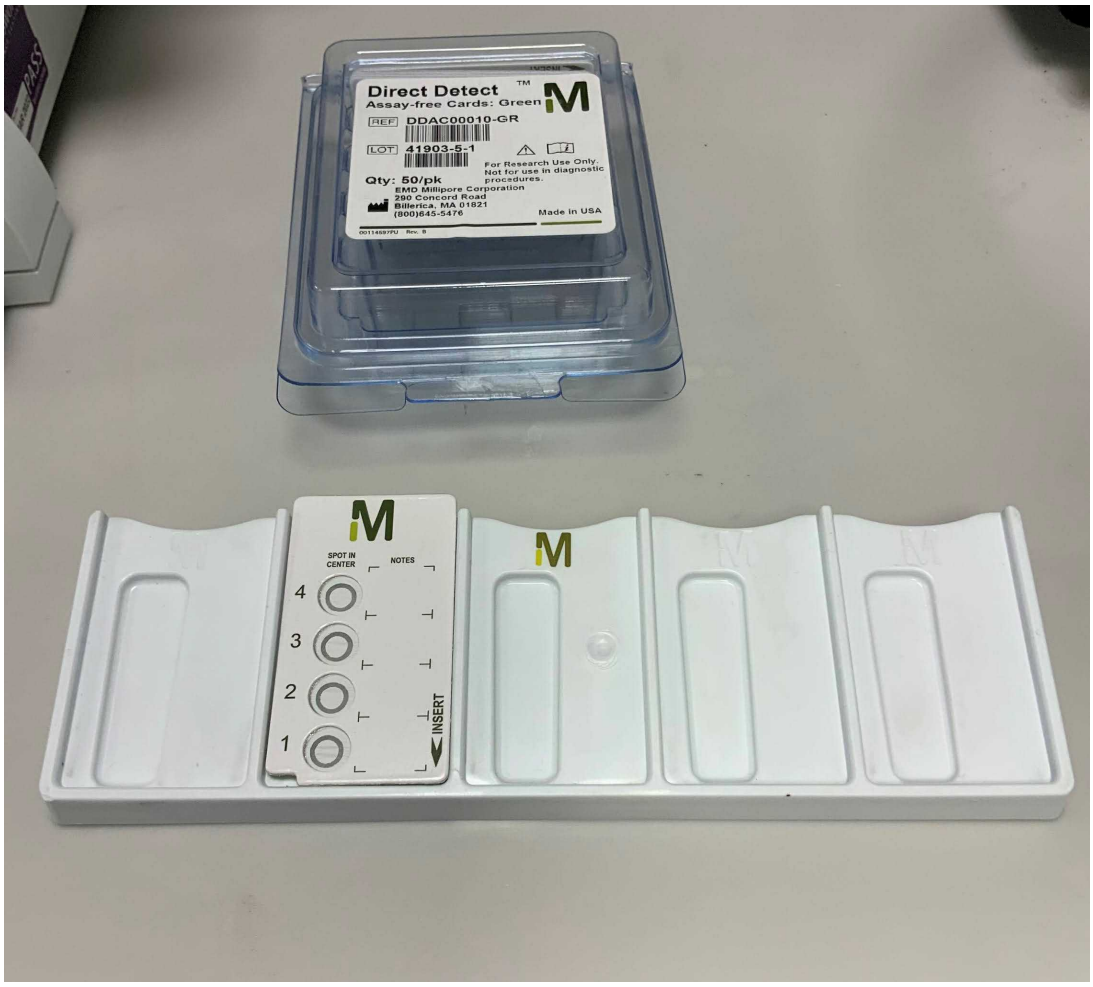


DD card in spotting tray.

2 Label the bottom membrane on the card for blank measurement.

3

Label a clean **0.75 mL** Eppendorf tube with “pool”. To it, add **2 µL** of each of your samples. When complete, vortex mix briefly. **A pooled sample should be representative of the batch you wish to measure, such as a set of replicates.**

4



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Pipette **2 µL** of blank into center of the membrane designated as the blank position.

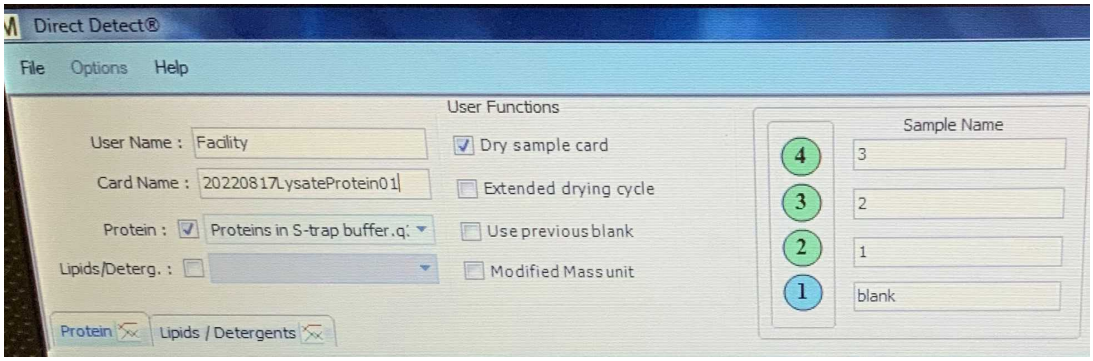
5

Pipette **2 µL** of your pooled sample to be analyzed into center of the three remaining spots.

Take care not to touch the membrane with the pipette tip, as this might tear the membrane.
Surface tension of the sample should be enough to pull it away from the tip onto the membrane.

**Software and measurement:** 5m

6 In the software, complete the fields as follows:
a. **User name** should be preset (Facility).
b. **Card name** – today's date, followed by your initials and the sample number.
c. **Protein** – use the drop down menu to select "proteins in S-Trap buffer.q3".
d. Ensure that the box marked "**dry sample card**" is ticked.
e. Do not tick the boxes marked "lipid", "extended drying cycle", "use previous blank", or "modified mass unit".
f. Give the 4 card positions a name, the position marked 1 in blue is the **blank**, while the other three should be in green, these are your three replicate measurements.




Data entry

7 Insert the Assay-free card vertically into the instrument card holder with the instrument and card arrows aligned.



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Make sure that the "M" writing side is facing the left. The instrument will move the card up and down and sound a tone. The green light illuminates to confirm proper insertion.


8 Click on the measure card button.

9 The sample concentrations will appear on the screen, along with the statistical analysis and spectrum plot. 5m

10 ⚠

After all four positions on the card have been read, the instrument will sound a tone. The card will rise to the initial insertion position. Remove the card and dispose of it.

- **Previous measurements may be found under the history tab.**
- The Direct detect is accurate for the measurement of protein lysates between



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[M]**0.3 mg/mL and [M]5 mg/mL .** If you obtain a reading that is higher than 5 mg.mL-1, it will not be accurate, because the calibration curve is not linear above this concentration. To obtain an accurate measurement, dilute your sample 1 in 5 and 1 in 10 in SDS S-Trap lysis buffer, and measure again in triplicate.



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