



Mar 04, 2021

Protein extraction and BCA assay from MCAS sorted cells

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Works for me

This protocol is published without a DOI.



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ABSTRACT

This protocol allows proteins extraction from cells sorted employing the MACS Adult Dissociation Brain kit (Miltenyi Biotec).

PROTOCOL CITATION

Daniel Manrique-Castano 2021. Protein extraction and BCA assay from MCAS sorted cells. [protocols.io](https://protocols.io/view/protein-extraction-and-bca-assay-from-mcas-sorted-bsz4nf8w)
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KEYWORDS

Protein extraction, Western Blot, BCA assay, Protein Measurement

LICENSE

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CREATED

Mar 04, 2021

LAST MODIFIED

Mar 04, 2021

PROTOCOL INTEGER ID

47900

GUIDELINES

Read the whole protocol before proceed.

MATERIALS TEXT

NP-40 based lysis buffer

NP-40 Surfact-Amps™ Detergent Solution Thermo Fisher

[M] 1 % (v/v) Scientific Catalog #85124

[M] 150 Milimolar (mM) NaCl

[M] 50 Milimolar (mM) Tris

BCA assay kit

Pierce™ BCA Protein Assay Kit Thermo Fisher

Scientific Catalog #23225

Step 7

BEFORE STARTING


Check reagent and microplate reader datasheets to select laser wavelength for measurement.

Protein Extraction

- 1 Following final centrifugation after MACS cells sorting, discard the supernatant and place the cells **On ice** to prevent protein degradation.
- 2 Prepare **NP-40 lysis buffer** and add it to the cells according to the number of expected proteins.

Usually **50 µl** - **100 µl** when protein concentration is expected to be low, or **200 µl** - **500 µl** when protein concentration is expected to be high.

- 3 Resuspend the cells by constant pipetting, and if it has not been done, transfer the cells to **1.5 mL** Eppendorf tubes.
- 4 It is recommended to perform **sonication** to facilitate protein lysis. Conditions may vary according to the sonication device. Usually, 20-40 seconds, 2 cycles, and middle power (50-70) are enough.

- 5   15m

If required, cells can be centrifugated at **13000 rpm, 20°C, 00:15:00** and the **supernatant must be taken**. The pellet is discarded.

- 6 Subsequently, **freeze the proteins** and keep them at **-20 °C** or **-80 °C**

BCA assay 30m 30s

- 7 The following procedure describes protein measurement by BCA assay using **Pierce™ BCA Protein Assay Kit Thermo Fisher Scientific Catalog #23225**. The protocol must be adapted if a different kit is employed.
- 8 Prepare or thaw **BSA standards** according to company protocol. Use NP-40 based lysis buffer as blank
- 9 **Working reagent** must be mixed (50:1, Reagent A:B). To calculate the total required amount, consider the number of replicates for BSA standards and cell samples. **200 µl** of working reagent re needed per sample/wheel.
- 10 In a 96-wheel plate, add **10 µl** (when the available sample is limited, range limited to 125-2000 ug/ML) or **25 µl** of **standards and samples** (achieved range 20-2000 ug/ml).

11 Immediately after, add  **200 µl** of **Working reagent** to each wheel.

12 

30m 30s

Place the plate in a **shaker** for  **00:00:30** , and subsequently, **incubate** the plate at  **37 °C** for  **00:30:00** .

If the microplate reader has a shaker option, placement in a shaker can be avoided and performed in this device.

13 Perform protein measurement according to reagent and microplate reader instructions.

Special attention should be paid to the wavelength for absorbance measurement. Some reagents work better at 562, while others at 750. Check kit datasheet.