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Brain Homogenization and MSD Protocol for Mouse Brain and Serum

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol details about the homogenization and MSD protocol of the mouse brain using various panel kits.

Attachments

PDF

320-681.pdf

54KB

Materials

Reagent, Kits and Equipment List

1.

Equipment

gentleMACS™ C Tubes	NAME
C Tubes	TYPE
gentleMACS™	BRAND
130-093-237	SKU
https://www.miltenyibiotec.com/US-en/products/gentlemacs-c-tubes.html#gref	LINK

2. RIPA Lysis and Extraction Buffer **Thermo Fisher Catalog #**89901

3. Halt PIC (ThermoFisher 1861279)

4.

Equipment

Direct Detect® Assay-free Cards	NAME
Assay-free Cards	TYPE
Direct Detect®	BRAND
DDAC00010-GR	SKU
https://www.emdmillipore.com/US/en/product/Direct-Detect-Assay-free-Cards,MM_NF-DDAC00010-GR?bd=1	LINK
Membrane cards used for protein quantitation in the Direct Detect® Infrared Spectrometer	SPECIFICATIONS

5. HCl (Millipore Sigma H1758-500ML)

6. NaOH (Millipore Sigma 415413-500ML)
7. HEPES **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**83264-500ML-F
8. Proinflammatory Panel 1 mouse **MESO SCALE DIAGNOSTICS, LLC. Catalog #**MSD K15048D
9. Cytokine Panel 1 Mouse Kit **MESO SCALE DIAGNOSTICS, LLC. Catalog #**K15245D
10. U-PLEX TGF-β Combo mouse **MESO SCALE DIAGNOSTICS, LLC. Catalog #**MSD K15242K-2
11.

Equipment

Octo Dissociator with Heaters	NAME
Octo Dissociator	TYPE
gentleMACS™	BRAND
130-096-427	SKU
https://www.miltenyibiotec.com/US-en/products/gentlemacs-octo-dissociator-with-heaters.html	LINK

12.

Equipment

Direct Detect Spectrometer	NAME
Spectrometer	TYPE
Direct Detect®	BRAND
DDHW00010-WW	SKU
https://www.emdmillipore.com/US/en/product/Direct-Detect-Spectrometer,MM_NF-DDHW00010-WW?bd=1	LINK

13. Meso Sector S 600 (MSD 1201)

Brain Homogenization Using Miltenyi gentleMACS Octodissociator

43m

1 Weight out brain using pre-chilled C-tube.

2 Use 2 mL RIPA (1x Halt PIC) per whole brain, 1 mL RIPA (1x Halt PIC) per half brain.

3 Homogenize brain using “protein_01_01” routine on Miltenyi gentle MACS Octodissociator.

3.1 Repeat homogenization routine if not fully homogenized.

4 Vortex briefly and incubate 00:30:00 On ice . 30m

5 Centrifuge at 3500 rpm, 4°C, 00:03:00 . 3m

6 Transfer homogenate to two 1.5 mL eppendorf tubes.

7 Vortex briefly and centrifuge at 14000 x g, 4°C, 00:10:00 . 10m

8 Transfer supernatant to new 1.5 mL eppendorf tubes

9 Perform 1:10 serial dilution.

9.1 Dilute using 10 µL sample to 90 µL RIPA (1x Halt PIC) to achieve 1:100 dilution. (1/2)

9.2 Dilute using 10 µL sample to 90 µL RIPA (1x Halt PIC) to achieve 1:100 dilution. (2/2)

10 Blot 2 µL diluted sample onto Direct Detect assay card (run duplicates).

11 Use 2 µL RIPA (1x Halt PIC) as blank.

12 Read assay card on Direct Detect Spectrometer.

13 Detectable range is 0.2 mg/mL to 5.0 mg/mL, dilute or concentrate as needed.

14 Average all readings for each sample to determine concentration.

Brain MSD Protocol Proinflammatory Panel 1 mouse and Cytokine Panel 1 mouse

15 Using concentration determined, calculate volume needed for 200 µg protein.

16 Use RIPA(1x PIC) to dilute 200 µg protein to 100 µL (2 µg/µL).

17 Combine 100 µL sample and 100 µL MSD diluent in U-bottom plate.

18 Load 50 µL diluted sample to MSD plate per duplicate.

19 Proceed with MSD assay protocol from manufacturer.

Brain MSD Protocol U-PLEX TGF-β Combo mouse

10m

20 Using concentration determined, calculate volume needed for 200 µg protein.

21 Use RIPA(1x PIC) to dilute 200 µg protein to 100 µL (2 µg/µL).

22 Load 100 µL sample into U-bottom plate.

23 Add 20 µL [M] 1 Mass Percent HCl into U-bottom plate and shake for 00:10:00 at 25 °C . 10m

24 Neutralize sample with 14 µL [M] 1.2 Mass Percent NaOH in [M] 0.5 Mass Percent HEPES.

25 Combine 100 µL treated sample and 100 µL MSD diluent in new U-bottom plate.

26 Load 50 µL diluted sample to MSD plate per duplicate.

27 Proceed with MSD assay protocol from manufacturer.

Serum MSD Protocol Proinflammatory Panel 1 mouse

28 Add 25 µL MSD Diluent to MSD Plate per duplicate.

29 Add 25 µL serum sample to MSD Plate per duplicate.

30 Proceed with MSD assay protocol from manufacturer.

Serum MSD Protocol Cytokine Panel 1 mouse

31 Add 37.5 µL MSD Diluent to MSD Plate per duplicate.

32 Add 12.5 µL serum sample to MSD Plate per duplicate.

33 Proceed with MSD assay protocol from manufacturer.

Serum MSD Protocol U-PLEX TGF-β Combo mouse

10m

34 Add 50 µL serum sample to U-bottom plate.

35 Add 10 µL [M] 1 Mass Percent HCl into U-bottom plate and shake for 00:10:00 at 25 °C. 10m

36 Neutralize sample with 7 µL [M] 1.2 Mass Percent NaOH in [M] 0.5 Mass Percent HEPES.

37 Add 25 µL MSD Diluent to MSD Plate per duplicate.

38 Add 25 µL treated serum sample to MSD Plate per duplicate.

39 Proceed with MSD assay protocol from manufacturer.