Brain Homogenization and MSD Protocol for Mouse Brain and Serum
Butterick Lab Updated 11/2/21 SS

Brain Homogenization Using Miltenyi gentleMACS Octodissociator

1. Weight out brain using pre-chilled C-tube
2. Use 2mL RIPA (1x Halt PIC) per whole brain, 1mL RIPA (1x Halt PIC) per half brain
3. Homogenize brain using "protein_01_01" routine on Miltenyi gentleMACS Octodissociator
    a. Repeat homogenization routine if not fully homogenized
4. Vortex briefly and incubate 30 minutes on ice
5. Centrifuge at 3500 RPM, 4℃ for 3 min
6. Transfer homogenate to 2 1.5mL eppendorf tubes
7. Vortex briefly and centrifuge at 14000xg, 4℃ for 10 min
8. Transfer supernatant to new 1.5mL eppendorf tubes
9. Perform 2 1:10 serial dilutions using 10µL sample to 90µL RIPA (1x Halt PIC) to achieve 1:100 dilution
10. Blot 2µL diluted sample onto Direct Detect assay card (run duplicates)
11. Use 2µL RIPA (1x Halt PIC) as blank
12. Read assay card on Direct Detect Spectrometer
13. Detectable range is 0.2-5.0mg/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

1. Using concentration determined, calculate volume needed for 200µg protein
2. Use RIPA(1x PIC) to dilute 200µg protein to 100µL (2µg/µL)
3. Combine 100µL sample and 100µL MSD diluent in U-bottom plate
4. Load 50µL diluted sample to MSD plate per duplicate
5. Proceed with MSD assay protocol from manufacturer

Brain MSD Protocol U-PLEX TGF-β Combo mouse

1. Using concentration determined, calculate volume needed for 200µg protein
2. Use RIPA(1x PIC) to dilute 200µg protein to 100µL (2µg/µL)
3. Load 100µL sample into U-bottom plate
4. Add 20µL 1M HCl into U-bottom plate and shake for 10 minutes at 25℃
5. Neutralize sample with 14µL 1.2M NaOH in 0.5M HEPES
6. Combine 100µL treated sample and 100µL MSD diluent in new U-bottom plate
7. Load 50µL diluted sample to MSD plate per duplicate
8. Proceed with MSD assay protocol from manufacturer

Serum MSD Protocol Proinflammatory Panel 1 mouse

1. Add 25µL MSD Diluent to MSD Plate per duplicate
2. Add 25µL serum sample to MSD Plate per duplicate
3. Proceed with MSD assay protocol from manufacturer

Serum MSD Protocol Cytokine Panel 1 mouse

1. Add 37.5µL MSD Diluent to MSD Plate per duplicate
2. Add 12.5µL serum sample to MSD Plate per duplicate
3. Proceed with MSD assay protocol from manufacturer

Serum MSD Protocol U-PLEX TGF-β Combo mouse

1. Add 50µL serum sample to U-bottom plate
2. Add 10µL 1M HCl into U-bottom plate and shake for 10 minutes at 25℃
3. Neutralize sample with 7µL 1.2M NaOH in 0.5M HEPES
4. Add 25µL MSD Diluent to MSD Plate per duplicate
5. Add 25µL treated serum sample to MSD Plate per duplicate
6. Proceed with MSD assay protocol from manufacturer

Reagent, Kits and Equipment List

1. C-tube (Miltenyi Biotec 130-093-237)
2. RIPA (ThermoFisher 89901)
3. Halt PIC (ThermoFisher 1861279)
4. Direct Detect Assay-free Cards (Millipore Sigma DDAC00010-GR)
5. HCl (Millipore Sigma H1758-500ML)
6. NaOH (Millipore Sigma 415413-500ML)
7. HEPES (Millipore Sigma 83264-500ML-F)
8. Proinflammatory Panel 1 mouse (MSD K15048D)
9. Cytokine Panel 1 mouse (MSD K15245D)
10. U-PLEX TGF-β Combo mouse (MSD K15242K-2)
11. gentleMACS Octodissociator (Miltenyi Biotec 130-069-427)
12. Direct Detect Spectrometer (Millipore Sigma DDHW00010-00)
13. Meso Sector S 600 (MSD 1201)