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

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SOP FOR MESOSCALE DISCOVERY ASSAYS

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ABSTRACT

SOP FOR MESOSCALE DISCOVERY ASSAYS

MATERIALS

- Assay diluent (included in kit)
- Tracer antibody diluent – 5940uL per plate(included in kit)
- Coating diluent – 3.3mL per plate (included in kit for certain assays)
- Biotinylated coating antibody(s) (included in kit for certain assays)
- Read buffer (included in kit)
- Calibrator(s) (included in kit)
- Tracer antibody(s) (included in kit)
- Plate(s) (included in kit)
- Adhesive seals – 3 per plate (included in kit)
- Strip tubes
- 1.5mL tubes
- Fast orbital shaker
- Multichannel pipette + tips with 25uL to 150uL capacity
- P-1000 and P-200 pipettes plus tips
- Wash buffer: PBS + 0.05% Tween-20 or bought from MSD
- Reagent reservoirs for multichannel pipetting

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Before Beginning:

1 Verify that all the kit contents are present in your order. Go through the included documentation to ensure all reagents were provided, and see which diluents are used for which purpose during the assay.

2 Get out all reagents to come to room temperature. If you're using a fresh bottle of diluent, get out the entire bottle. You'll want to aliquot this into single-use tubes after completion of the assay.

3 For plasma/serum, aliquot each sample into strip tubes at an appropriate volume/dilution for the assay at hand. Keep track of dilution factors to compute the actual concentration upon completion of the assay. This is recommended to be done the same day to avoid unnecessary freeze-thaws of samples, but it can be done ahead of time and stored in the freezer.

3.1 For the Mouse Pro-Inflammatory kit, use 30uL of plasma and 90uL of Diluent 41 for a 1:4 dilution.

3.2 For the R-Plex CRP assay, 5uL of plasma was diluted into 45uL of Diluent 100 (1:10). 5uL of this was diluted into another 45uL of Diluent 100. 15uL of this 1:100 dilution stock was diluted into 135uL of Diluent 100 for a final dilution of 1:1000.

3.3 For the R-Plex LCN2 assay, dilute 5uL of plasma into 45uL of Diluent 100, then dilute 15uL of this solution into 135uL of Diluent 100, for a final dilution of 1:100.

3.4 Dilutions of plasma for other assays should be empirically determined.

3.5 Protein extracted from solid samples (e.g., frozen tissue, stool) should be diluted in MSD lysis buffer to an appropriate concentration for the assay at hand, also empirically determined. Use of solid samples will not be described here. MATERIALS:

Protocol:

4 Coat the plate if needed for the assay. If this is not needed, skip to step 2.

4.1 For 1 plate, add 200uL biotinylated capture antibody into 3.3mL of Diluent 100. Scale volumes up as needed for multiple plates.

4.2 Dispense 25uL into each spot. Use reverse pipetting to avoid bubbles.

4.3 Incubate for 1hr at RT at 700rpm.

5 Wash 3x150uL with wash buffer with reverse pipetting to avoid bubbles.

6 During coating and washing, prepare calibrators as specified in the included booklet, leaving a blank calibrator tube as specified. Make sure to use the appropriate diluent. Don't change tips between serial dilutions to ensure.

7 Load the plate:

7.1 Add 50uL of prepared samples or calibrators in duplicate to each well. Use reverse pipetting to avoid bubbles.

7.2 Incubate at RT at 700rpm. Incubation times vary between assays – check included booklet.

8 Wash 3x150uL with wash buffer with reverse pipetting to avoid bubbles.

9 Prepare detection antibody immediately before use:

9.1 For 1 plate, add 60uL of tracer antibody into 5940uL of tracer antibody diluent. Scale volumes up as needed for multiple plates.

10 Add tracer antibody to plate:

10.1 Dispense 50uL of solution to each well. Use reverse pipetting to avoid bubbles.

10.2 Incubate at RT at 700rpm. Incubation times vary between assays – check included booklet.

11 Wash 3x150uL with wash buffer with reverse pipetting to avoid bubbles.

12 Dilute read buffer if specified by assay.

13 Dispense 150uL read buffer to each well with reverse pipetting.

14 Analyze on MSD instrument as available. If reading multiple plates, ensure that lag time between read buffer application and reading is held constant between plates.