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Making Cancer History®

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Please remember to cite the CCSG grant that supports the ORION core (NIH P30CA016672) and mention the ORION Core lab in all your publications.

ORION Core Standard Operating Procedure		
Tissue Lysate Preparation for Luminex Soluble Factor Analysis		
Date Prepared: 08/29/2018	**Date Revised:** n/a	**Version:** 2
Submitted by: Karen Millerchip	**Approved by:** Dr. Cara Haymaker	**Category:** Luminex

1. INTRODUCTION

1.1. The Luminex xMAP technology can be used to identify and quantitate intracellular proteins from cultured cells or solid tissues. To do this, cells or tissues need to be lysed in a pH-neutral, low-detergent lysing buffer and total protein concentrations for each sample within an experiment should be normalized.

2. SAFETY PRECAUTIONS

2.1. The required precautions and procedures outlined for compliance with OSHA's Bloodborne Pathogens Standard should be followed when handling human tissues. Wear appropriate personal protective equipment (PPE) such as labcoat and gloves when performing the assay.

3. MATERIALS/EQUIPMENT

- ProcartaPlex Cell Lysis Buffer, 20ml and stored at -20°C (Life Technologies EPX-99999-000)
- PMSF Protease Inhibitor, 100mM (Boston Bioproducts BP481)
- gentleMACS Dissociator (Miltenyi 130-093-235)
- gentleMACS M Tubes (Miltenyi 130-093-236)
- DC Protein Assay Kit I (Bio-Rad 500-0111)
    - 250 ml REAGENT A, an alkaline copper tartrate solution
    - 2000 ml REAGENT B, a dilute Folin Reagent
    - 5 ml REAGENT S
    - bovine gamma globulin standard, lyophilized and stored at 4°C
- Pipettors P10, P20, P200, P1000
- Microcentrifuge, refrigerated and set to 4˚C
- Benchtop centrifuge, refrigerated and set to 4˚C
- Microcentrifuge tubes
- Microtiter plate
- Microplate reader set to 750nm (example: Bio-Tek Synergy HT)

4. REFERENCES

- Affymetrix/eBioscience Bulletin 30.07.15: "Preparation of Tissue Homogenate."
- Invitrogen/Thermo Fisher protocol 29.11.2016 (02): "Cell Lysis Buffer."
- Miltenyi Biotec protocol: "Homogenization of tissue for protein extraction."
- Bio-Rad Manual: "DC Protein Assay Instruction Manual."
- Bio-Rad Bulletin 1770: "Total Cellular Protein Determination Using the DC Protein Assay."
- Bio-Rad Tech Note 1069: "Colorimetric Protein Assays."
- Bio-Rad Bulletin 6837 Ver A: "DC Protein Assay Quick Guide."

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5. PROCEDURE

5.1. Prepare ProcartaPlex Cell Lysis Buffer:
- 5.1.1. If buffer will be continually used, it is recommended that the cell lysis buffer be kept at 4°C for 1-2 weeks. For longer periods of time, buffer should be stored at -20°C.
- 5.1.2. Thaw cell lysis buffer at 24-30°C, mix by inverting bottle.
- 5.1.3. Chill cell lysis buffer on ice and add a final concentration of 1mM PMSF just prior to use. Dilute 100mM stock PMSF solution 1:100 in the ProcartaPlex Cell Lysis Buffer.

5.2. Weigh tissue.
- 5.2.1. Liver, kidney, spleen, heart, lung, brain, skin, muscle, or intestine will work for this lysis process. Very hard material such as bone, cartilage, or mouse tail may damage the M Tube.
- 5.2.2. Use 500µl ProcartaPlex Cell Lysis Buffer per 100mg of tissue. The sample volume should be between 300µl and a maximum of 10ml of lysis buffer.

5.3. Obtain a Miltenyi M Tube (orange cap):
- 5.3.1. Pipette appropriate amount of ice-cold lysis buffer into the M Tube.
- 5.3.2. Transfer tissue sample into the lysis buffer.
- 5.3.3. Tightly close M Tube and turn it upside down in one quick move ensuring that the tissue reaches the rotor.
- 5.3.4. Attach M Tube upside down onto the sleeve of the gentleMACS Dissociator so that you hear a “click.”
- 5.3.5. Run the gentleMACS Program **Protein_01**.
- 5.3.6. Once the program is complete, detach M Tube from the gentleMACS Dissociator.
- 5.3.7. In a refrigerated benchtop centrifuge, quick-spin the sample at 500xg for 1 minute at 4°C to collect the sample material at the bottom of the tube.
- 5.3.8. Transfer the sample to a microcentrifuge tube for a hard spin.

5.4. Centrifuge the lysate in a microcentrifuge at 16,000xg for 10 minutes at 4˚C.

5.5. Transfer the supernatant to a new microcentrifuge tube.

5.6. Measure total protein concentration using the Bio-Rad DC Protein Assay Kit I, a Lowry-based colorimetric assay suitable for samples containing detergent. Set up the microplate version of the assay to save sample volume.
- 5.6.1. Prepare working reagent A: add 20µl of reagent S to each ml of reagent A that will be needed for the run.
- 5.6.2. Prepare 5 dilutions of bovine gamma globulin standard from a top standard of 1.5 mg/ml protein reconstituted in ProcartaPlex Cell Lysis Buffer. A standard curve should be prepared each time assay is run. Reconstituted protein standard can be stored at -20 °C for 6 months.
- 5.6.3. Pipet 5µl of standards and samples into a clean, dry microtiter plate.
- 5.6.4. Add 25µl of working reagent A into each well.
- 5.6.5. Add 200µl reagent B into each well. If microplate reader has a mixing function available, place plate in reader and let the plate mix for 5 seconds. If not, gently agitate the plate to mix the reagents.
- 5.6.6. After 15 minutes, absorbances can be read at 750nm. The absorbances will be stable for about 1 hour.
- 5.6.7. Determine protein concentration by plotting absorbance vs concentration of known standards. Use the resulting curve to determine the concentration of unknown proteins based on their absorbance.

5.7. Adjust all samples to equal protein concentration by diluting to 10mg protein/ml with ice-cold ProcartaPlex Cell Lysis Buffer.

5.8. Store samples at -80°C until ready to assay by Luminex.