

<b>ORION Core Standard Operating Procedure</b>		
<b>Cell Culture Supernatant Preparation for Luminex Soluble Factor Analysis</b>		
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## 1. INTRODUCTION

1.1. The Luminex xMAP technology can be used to identify and quantitate secreted proteins from cultured cells.

## 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

- Benchtop centrifuge, refrigerated and set to 4°C
- Benchtop centrifuge plate carriers
- Optional: Thermo Scientific Nunc Sealing Tape, Fisher #12-565-398
- Optional: Thermo Scientific Nunc 96-Well Polypropylene Storage Microplates, Fisher #12-565-436
- Optional: Polypropylene Microtubes (Eppendorf tubes)
- Single or Multichannel pipettor

## 4. REFERENCES

- Bio-Rad Manual: “The Bio-Plex Success Guide for Multiplex Immunoassays.” Bulletin 6458 Rev B.
- Invitrogen: “Sample Prep Guide: Proper sample handling for immunoassays.”

## 5. PROCEDURE

- 5.1. Centrifuge cell culture plate with the lid on in a plate carrier at 1,000 x g for 15 minutes at 4°C.
- 5.2. Carefully pipet off supernatant without touching the bottom of the well and without mixing. Only pipet off top half of the supernatant to avoid pulling up cellular debris.
- 5.3. Aliquot supernatant into low protein-binding polypropylene Eppendorf tubes or polypropylene microplates.
- 5.4. If using a microplate, seal tightly with a plate sealer that can withstand -80°C temperatures.
- 5.5. Store supernatants at -80°C until ready to use.