

Cell-Mate3D™ Protein Isolation Protocol

Use this protocol to extract and quantify protein from cell-embedded Cell-Mate3D™ matrix.

REQUIRED EQUIPMENT

- Pestle for 2mL microfuge tubes (USA Scientific #1415-5390)
- 1.5mL microfuge tubes
- Styrofoam Box
- Liquid Nitrogen
- Cryogenic Gloves
- Microplate Reader/Spectrophotometer
- Ice/ice bucket
- Kimwipe
- Sonicator
- Refrigerated Microcentrifuge

RECOMMENDED REAGENTS

- NP40 cell lysis buffer (Thermo Scientific #FNN0021)
- PMSF (Sigma #P7626)
- Protease inhibitor cocktail (Sigma #P2714)
- Pierce BCA Protein Assay Kit (Thermo Scientific #23225)

PROTOCOL

1. Prepare NP40 lysis buffer solution to the desired concentrations or 1mM PMSF, 1X protease inhibitor, according to manufacturer's guidelines.
2. In a thermo-stable container, fill a 1.5mL microfuge tube with liquid nitrogen and chill the pestle.
3. Remove the Cell-Mate3D™ sample from culture and briefly wash in PBS twice. Blot the Cell-Mate3D™ sample dry with kimwipes to remove excess fluid.
4. Working quickly, cut the Cell-Mate3D™ into 2-3mm³ pieces and transfer into the 1.5mL microfuge tube filled with liquid nitrogen.

5. When the Cell-Mate3D™ sample is frozen solid it will sink to the bottom of the tube. When the liquid nitrogen evaporates, grind into a fine powder with the pestle. Flick the tube occasionally for homogenous grinding.
6. Lyse the finely ground sample with 150µL NP40 lysis buffer. Mix well.
7. Sonicate on ice at 30% amplitude for a total of 8 seconds with 1 second intervals.
8. Incubate on ice for 20 minutes.
9. Spin down the sample in a microcentrifuge at 17,000g at 4°C for 10 minutes.
10. Carefully transfer supernatant (containing the isolated protein) to a new, clean, 1.5mL tube. Do not disrupt the pellet.
11. Quantify your protein isolate with the BCA protein assay kit.

Safety Disclaimer:

Only competent and trained personnel using appropriate personal protective equipment and working within a controlled environment should handle all chemicals and perform the protocol described herein. Prior to performing this protocol, users should review appropriate safety information, including the manufacturers MSDS, related to the components used in this protocol. Bioactive Regenerative Therapeutics, Inc. shall not be held liable for any loss, injury or damage as a result from the use of this protocol.