

Protocol for protein extraction

Analysis of protein expression in cells cultured in Biomimesys® is performed using standard techniques of protein extraction with some modifications.

It is advisable to work on ice.

- Prepare the usual lysis buffer.

For example: Tris 50mM pH8 + 1mM MgCl₂ + 1mM EDTA + 1% Triton + 50mM NaCl + protease inhibitor.

- Adjust the pH of the buffer to 7.4.
- Cool down the centrifuge rotor for microtubes to 4°C.
- Prepare 1 microtube containing PBS (approximately 1ml) and 3 tubes (15ml Falcon) filled with 2ml of DMEM without FCS at 37°C.
- Pick the hydrogels with fine forceps and dip:
 - Once in PBS (2 hydrogels/tube).
 - Followed by once in each tube containing DMEM without serum (FCS) for 2mins per tube and turn the tubes 3 times (with the lid closed).
- Rinse both hydrogels one last time in 1mL cold PBS before placing them at the bottom of a microtube in 100µL of cold lysis buffer.
- Vortex twice for about 5 seconds.
- Incubate for 30mins, vortex gently every 5 minutes.
- Finally, centrifuge the tubes at 4°C for 10mins at 13,000g.
- Collect the supernatant and proceed with protein assay or store at -80°C.