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 hydroscaffold with fine forceps and dip:
  - Once in PBS (2 hydroscaffold /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 hydroscaffold 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.

Contact Information

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PROTEIN EXTRACTION

INTRODUCTION

Measurement of protein expression (Western blot) within a cell culture by protein extraction. The amount of protein produced by cells can be measured by antibody detection. Western blot can be used to detect the protein in a semi-quantifiable manner by interaction with a specific antibody.

METHOD

Follow standard protocols for protein extraction, the lysis buffer can be added directly to the hydroscaffold.

KIT TESTED WITH RESULTS
For protein extraction:

Kits – HT29 cells	Time	Yield/pastille
Standard protein extraction	Day 7	50-80µg
	Day 14	70-90µg

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