

RASTRUM

Protocol

Protein Extraction & Quantification from RASTRUM™ 3D Cell Models



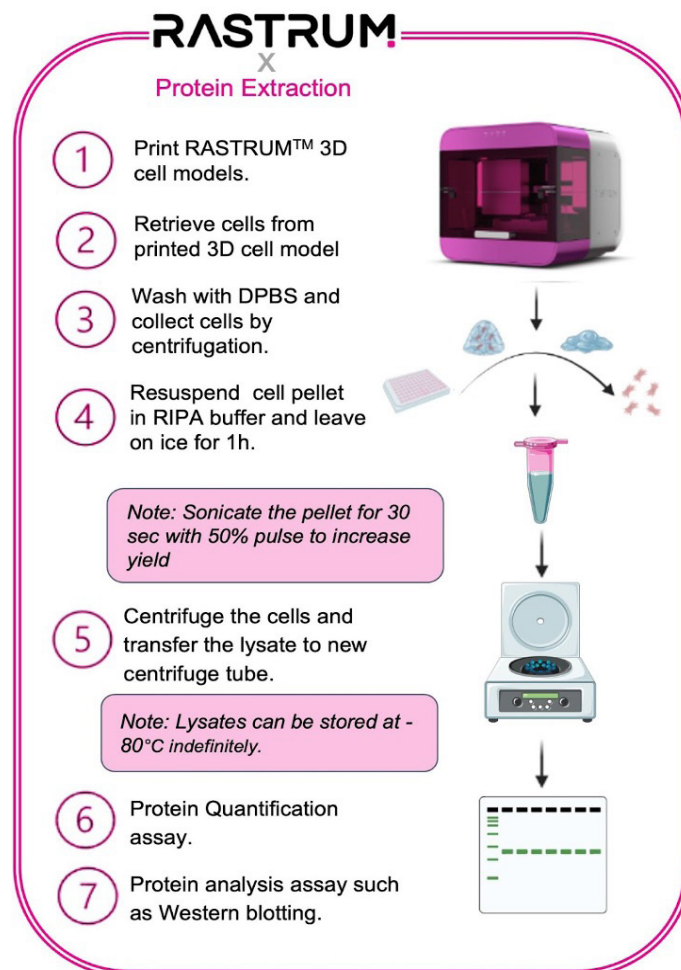
Introduction

Protein analysis involves a broad range of experimental techniques for the detection, identification and characterisation of proteins – each requiring the extraction of protein and quantification of their levels. This protocol details how to extract and quantify proteins from RASTRUM 3D cell models.

Equipment and reagents required, but not provided

- RASTRUM 3D cell models
- Dulbecco’s Phosphate-Buffered Saline (DPBS)
- RIPA Lysis Buffer (Thermo 89900; or similar)
- Protease Inhibitor (Thermo 78430; or similar)
- Phosphatase Inhibitor (Thermo 78420; or similar)
- *Recommended:* DC Protein Assay (Bio-Rad 5000111)

Graphical Protocol



Protocol

1. Retrieve cells from RASTRUM 3D cell models according to our [Cell Retrieval protocol](#).
2. Wash the retrieved cells twice with cold DPBS. Collect the cells by centrifugation at 300 g for 5 min at 4°C.
3. Vigorously resuspend the cell pellet in 25 µL of RIPA Buffer and leave on ice for 1 h.
Note: We recommend adding protease and phosphatase inhibitors to the RIPA buffer to prevent the degradation and dephosphorylation of protein targets.
Note: To increase yields, sonicate the pellet for 30 seconds with 50% pulse.
4. Centrifuge the cells at 12,000 g for 20 min at 4 °C and transfer the lysate to a new centrifuge tube.
Note: Lysates can be stored at -80°C almost indefinitely.
5. Proceed with protein quantification assay according to the manufacturer's instructions (tested compatible with DC Protein assay).
6. Proceed with protein analysis assay, such as Western Blotting with 20-40 ng of protein (Figure 1).

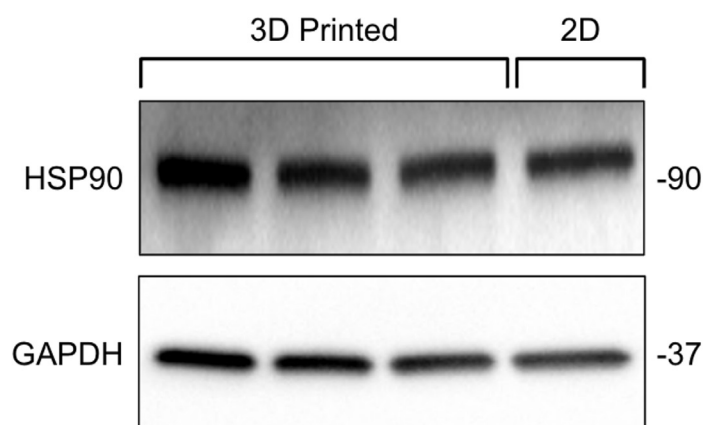


Figure 1: Western blot detection of HSP90 and GAPDH in RASTRUM 3D cell models following cell retrieval and 2D controls (30 ng of protein added for each sample).



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