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Total Protein Extraction from Cells Cultured in Alvetex® in 3D

Introduction

The following protocol outlines a method for the extraction of total protein from cells cultured in alvetex®. Example data was obtained using this protocol to extract protein from HepG2 hepatocytes cultured in alvetex® for 7 days in 6-well inserts (AMS.AVP004-32) in Well Insert Holder in Deep Petri Dish (AMS.AVP015-2) format.

Method:

1. Prepare lysis buffer: 1 % v/v Igepal® CA-630 (Sigma, Product No. I3021), 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM $MgCl_2$, and Complete Mini Protease Inhibitor Cocktail (Roche Diagnostics, 11 836 153 001).

   Note: The Protease Inhibitor Cocktail is available in two forms, with or without EDTA lysate, see manufacturer instructions for recommendation.
2. Remove alvetex® disc from culture format (e.g. well insert or 12-well multiwell plate) and wash by gentle immersion in PBS (e.g. in a Petri dish). Transfer into a 1.5 ml tube.
3. Add 1 ml of lysis buffer and vortex for 10 seconds. Place on ice for 30 minutes with vortexing (10 sec.) every 5 minutes.
4. Clarify lysates by centrifugation at approx 15,000 rpm for 3 minutes and place supernatant into a fresh tube.
5. The samples can be frozen at this stage (e.g. at -80°C), for use at a later date.
6. Determine total protein content of samples using a protein determination assay (e.g. Bio-Rad protein assay).

Example: Extraction of Total Protein from HepG2 Hepatocytes Cultured in Alvetex® in 3D

Cell Culture:

HepG2 cells (ATCC, HB-8065) were routinely maintained in T-75 flasks. HepG2 complete media consisted of: MEM media (Gibco, 21090) supplemented with 10 % v/v FBS, 2 mM L-glutamine and 100 U/ml Penicillin/Streptomycin. Cells were seeded onto alvetex® discs in 6-well inserts (AMS.AVP004-32) in well insert holders in deep Petri dishes (Product No. AMS.AVP015-2), at a density of 1x 10^6 cells in 150 µl media per insert. After settling for 3 hours in an incubator (5 % CO_2, 37°C) complete media was carefully added (70 ml per Petri dish). Cultures were maintained for 7 days, with a single media change on day 5. After 7 days, well inserts were dismantled and cultures were processed according to the protocol described above.

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Protein analysis results:

Determination of relative protein concentration was made using Protein Assay Reagent (Bio-Rad, 500-0006;). A dilution series of BSA was used for the generation of a standard curve (Figure 1. inset). Approximately 1 milligram of protein was isolated per disc (Table 1).

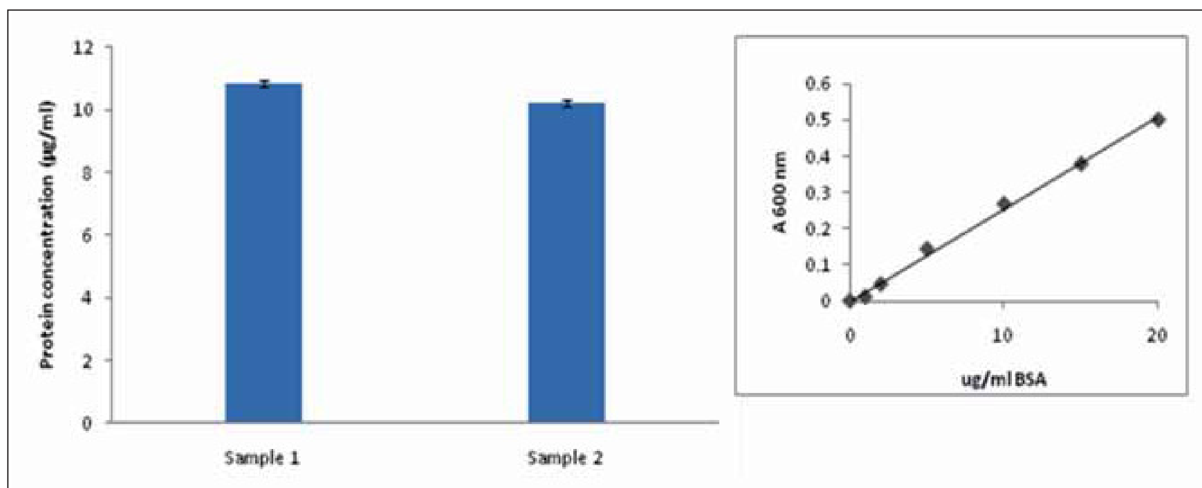

Figure 1. *Estimation of protein concentration (relative to BSA) in extracts from alvetex® cultures of HepG2 hepatocytes. Extracts were assayed at a dilution of 1 in 100. Data from two biological replicate cultures are shown; each sample was read in triplicate.*

	Sample 1	Sample 2
Protein yield (mg/ml)	1.08 (±0.01)	1.02 (±0.01)

Table 1. *Approximate protein yields in extracts from alvetex® cultures of HepG2 hepatocytes, using BSA as a standard reference. Values shown represent the mean of triplicate readings per sample (±SD).*

Protein extracts were also analysed by SDS-polyacrylamide gel electrophoresis and western blotting (Figure 2). Protein extracts from two biological replicate cultures produced well-resolved and highly reproducible SDS-PAGE profiles as determined via Coomassie Brilliant Blue R staining. Western blotting demonstrated that the constitutive protein β-actin was both intact and equally loaded in both sample lanes.

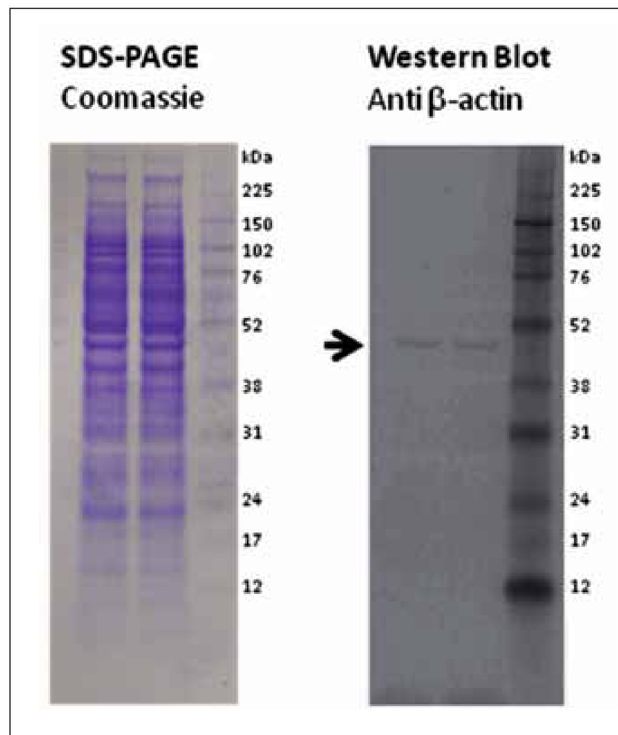

Figure 2. *SDS-polyacrylamide gel electrophoresis and western blot analysis of protein extracts from alvetex® cultures of HepG2 hepatocytes. Polyacrylamide gels (NuPAGE 10% Bis-Tris, Invitrogen, NP0302) were loaded with 20 μg protein. Resolved gels were stained with Commassie Brilliant Blue R (left) or blotted to PVDF membrane for western analysis (right). The western blot was probed for the constitutive protein β-actin (arrow). Data from two biological replicate cultures are shown.*

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