

Introduction

The following protocol outlines the use of a commercial extraction kit (Qiagen RNeasy® kit, 74104) for the isolation of total RNA from cells cultured in alvetex®. Example data was obtained using this protocol to extract RNA 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. Wash alvetex® culture by gentle immersion in PBS using flat-ended forceps and transfer to a clean 12-well plate.
2. Lyse cells by adding 600 µl Qiagen RNeasy® kit lysis buffer RLT per well.
3. Place on a rotating platform (100 rpm) for 10 minutes at room temperature.
4. Homogenise the lysate by passage up and down through a 20-gauge needle 10 times using a sterile plastic syringe.
5. Add an equal volume of 70 % ethanol to the homogenised lysate and pipette up and down 10 times to mix.
6. Transfer sample (up to 700 µl at a time) to an RNeasy® spin column in a 2 ml collection tube. Close cap and microcentrifuge for 15 seconds at 8000 x g. Discard flow-through. Repeat for remaining volume of lysate. Optional: On-column DNase digestion can be performed at this point (steps 6 a-d). Alternatively proceed to step 7.
 - a. Add 350 µl Buffer RW1 to the column. Close cap, and microcentrifuge for 15 seconds at 8000 x g to wash the membrane. Discard flow-through.
 - b. Prepare DNase 1 reagent by adding 10 µl DNase 1 stock solution (prepared according to manufacturer's instructions) to 70 µl Buffer RDD. Mix by gentle pipetting or gentle tube inversion.
 - c. Add the 80 µl diluted DNase 1 reagent to the column membrane and incubate at room temperature for 15 minutes.
 - d. Add 350 µl Buffer RW1 to the column. Close cap and microcentrifuge for 15 seconds at 8000 x g. Discard flow-through. Proceed to step 8.
7. Add 700 µl Buffer RW1 to the column. Close cap and microcentrifuge for 15 seconds at 8000 x g, reusing the collection tube. Discard flow-through.
8. Add 500 µl Buffer RPE (appropriately diluted with ethanol according to manufacturer's instructions) to the column. Close cap and microcentrifuge for 15 seconds at 8000 x g. Discard flow-through.

9. Add a further 500 µl Buffer RPE to the column. Close cap and microcentrifuge for 2 minutes at 8000 x g. Discard flow-through.
10. Transfer column to a clean 2 ml collection tube. Close cap and microcentrifuge at full speed for 1 minute.
11. Transfer column to a clean 1.5 ml collection tube and add 50 µl RNase-free water onto the column membrane. Close cap and leave at room temperature for 5 minutes. Microcentrifuge for 1 minute at 8000 x g to elute RNA.

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

Cell Culture details:

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 (AMS.AVP015-2), at a density of 1×10^6 cells in 150 µl media suspension per insert. After settling for 3 hours in an incubator (5 % CO₂, 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.

RNA analysis results:

RNA samples were assessed for quantity and quality by spectrophotometric (Table 1) and agarose gel (Figure 1) analyses.

	Sample 1	Sample 2
RNA yield (µg/ml)	185.1 (±0.044)	182.7 (±0.470)
A260/A280	2.123 (±0.012)	2.117 (±0.006)

Table 1. RNA yield and purity analysis. RNA samples from duplicate HepG2 alvetex® cultures were diluted 5-fold in 10 mM Tris-HCl, pH 7.0. Values shown represent the mean of triplicate readings per sample (±SD), taken on a NanoDrop 1000 spectrophotometer (Thermo Scientific).

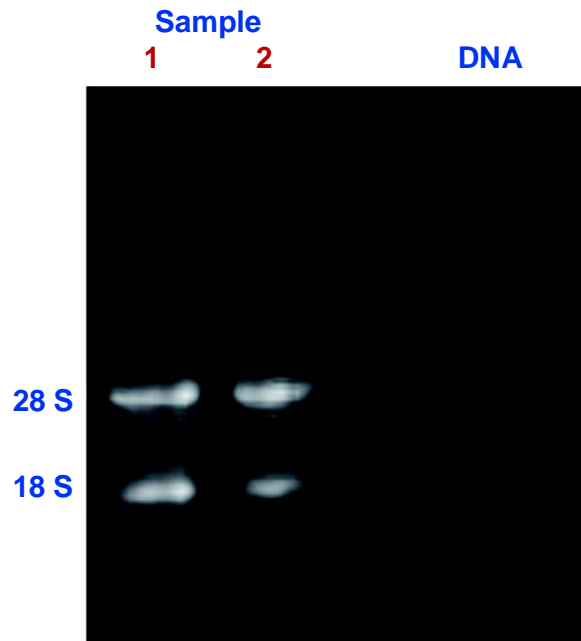


Figure 1. Agarose gel RNA profile from duplicate HepG2 alvetex® 3D cultures (Samples 1 and 2). 28S and 18S rRNA bands are clearly visible confirming RNA integrity (1% agarose gel; visualisation via ethidium bromide staining; 1 kb molecular weight ladder: Promega, G571A).

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