Purification of DNA from 3D cell cultures using the Maxwell® RSC Cultured Cells DNA Kit

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Materials Required

- Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 3D cell culture

Protocol: *Maxwell® RSC Culture Cells DNA Kit Technical Manual* #TM477 and *Maxwell® RSC Methods Installation Technical Manual* #TM435, available at: **www.promega.com/protocols**, or e-mail: **techserv@promega.com**

Introduction

In vitro mammalian cell culture provides a defined platform for investigating cell and tissue physiology and pathophysiology. Traditionally, cell populations have been cultured on two-dimensional (2D) substrates such as tissue culture polystyrene (TCPS) or the surface of tissue analogs. Mammalian cells growing in a monolayer, however, only establish cell-cell contacts at the periphery where conditions are remarkably distinct from real in vivo situations. Furthermore, it is known that many aspects of the cell microenvironment play a major role in cellular morphology, physiology, metabolism and the response to external stimuli. Three-dimensional (3D) cell cultivation has therefore become an essential and popular platform for researchers looking to more accurately mimic the native cell environment.

The Maxwell® RSC Cultured Cells DNA Kit, in combination with the Maxwell® RSC Instrument, provides a simple method for efficient, automated purification of genomic DNA (gDNA) from mammalian and bacterial cell culture. This application note describes the protocol for using the Maxwell RSC® Cultured Cells DNA Kit with the Maxwell® RSC Instrument to purify DNA from 3D cell cultures.

Protocol Methods

1. Place the Maxwell® cartridge to be used in the deck tray with the printed side facing away from the elution position, which is the numbered side of the tray.
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing the cartridge in the instrument.

   Note: If you are processing fewer than 16 samples, center the cartridges on the deck tray.
3. Transfer the cultured cell samples to well #1 of each cartridge and thoroughly mix the cultured cell samples into the lysis buffer by pipetting at least 10 times. (Well #1 is the well closest to the printed side and furthest from the elution tube.)
   a. For Ultra-Low Attachment (ULA) Cell Culture, load spheroids directly into the first well of the Maxwell® cartridge in ≤400µL of culture media using a wide bore pipette tip.
   b. For Matrigel® Matrix cell culture, remove media from surface of Matrigel® Matrix. Using a wide bore pipette tip gently remove the Matrigel® Matrix and transfer it to the first well of the Maxwell® cartridge.
4. Place one plunger in well #8 of each cartridge. (Well #8 is the well closest to the elution tube.)
5. Place an empty elution tube into the elution tube position for each cartridge. Add 100µl of Elution Buffer to the bottom of each elution tube.

   Note: Use only the Elution Tubes (0.5ml) provided with the kit; other tubes may be incompatible with the Maxwell® RSC Instrument.

6. Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 for detailed information. To run the RSC Cultured Cells DNA protocol, the Maxwell® RSC Cultured Cells DNA method must be installed on your instrument. The method is available at: **www.promega.com/resources/tools/maxwellrscmethod/**. See *the Maxwell® RSC Methods Installation Technical Manual* #TM435 for instructions.
7. Follow the instrument run instructions in the Maxwell® RSC Cultured Cells DNA Kit Technical Manual #TM477

Results

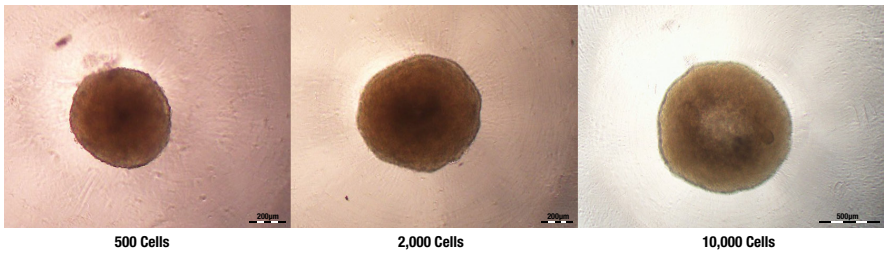

Figure 1. Images of spheroids grown in Corning® Ultra-Low Attachment Plates. HCT116 cells were plated at 500, 2,000 and 10,000 cells per well in Corning® Ultra-Low Attachment 96-well plates. Cells formed spheroids over 4 days of growth and were then imaged and harvested for DNA purification.

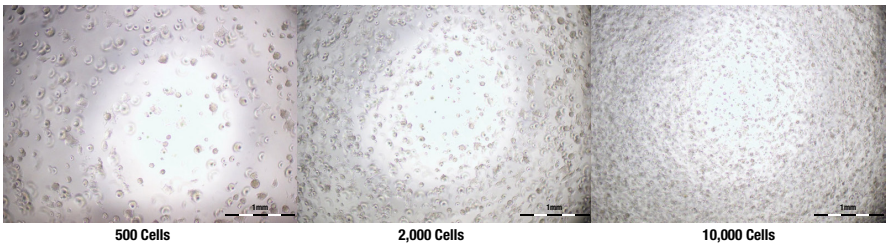

Figure 2. Images of cells grown in Matrigel® Matrix. HHCT116 cells were plated at 500, 2000 and 10,000 cells per well in 50µl of Matrigel® Matrix following standard methods. Cells formed small spheroids over 4 days and were then imaged and harvested for DNA purification.

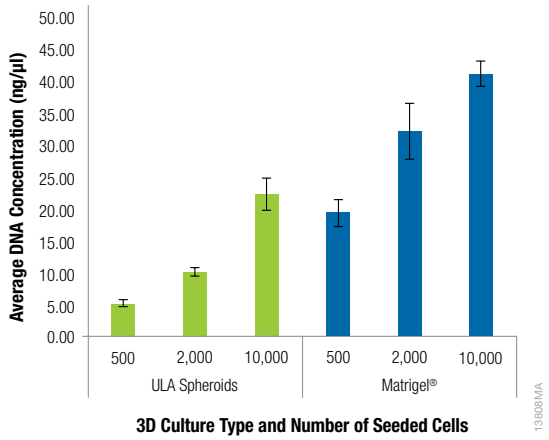

Figure 3. Quantification of purified DNA from 3D cell cultures. Using the Maxwell® RSC Cultured Cells DNA Kit with the Maxwell® RSC Instrument, DNA was purified in triplicate from two 3D cell culture methods, each seeded at densities of 500, 2,000 and 10,000 cells. Purified DNA was quantified in triplicate by fluorescent quantification (QuantiFluor® ONE dsDNA System).

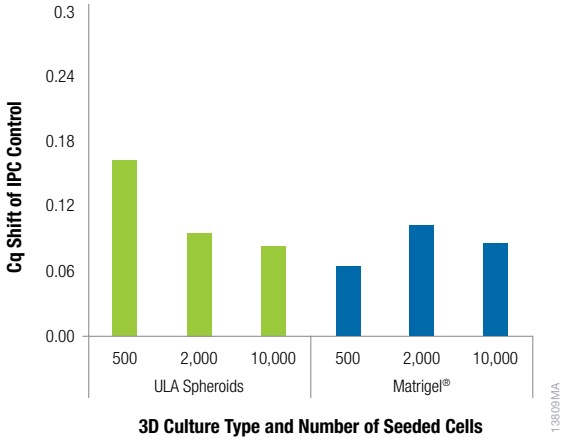

Figure 4. Test for amplification inhibition. Purified DNA was amplified using qPCR and inhibition measured using an internal positive control (IPC). A C_q shift of ≥0.3 between average of standards and samples is considered significant, indicating possible inhibition. None of the samples showed significant inhibition.

Summary

The Maxwell® RSC Cultured Cells DNA Kit and Maxwell® RSC Instrument provide a simple and rapid method to purify high quality DNA from 3D cell culture. DNA is purified at sufficient concentrations (Figure 3) from even low cell densities and is free of amplification inhibitors (Figure 4) that would negatively impact sensitive downstream assays.

Ordering Information

Product	Size	Cat.#
Maxwell® RSC Cultured Cells DNA Kit	48 preps	AS1620

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