Promega

Product Application

DNA isolation from 3D microtissues or cells embedded in Matrigel® using the Maxwell® RSC Cultured Cells DNA Kit

To isolate high quality, amplifiable DNA from 3D microtissues using the Maxwell® RSC Cultured Cells DNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620)

Analyses: Dye-based quantitation, qPCR

Sample Type(s): 3D microtissues in ULA plates or Matrigel®

Input: One 3D microtissue or cells embedded in Matrigel®

Materials Required:

- Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Corning® Matrigel® Basement Membrane Matrix (Corning, Cat.# 354248) or similar
- Corning® 96 Well Black Clear Round Bottom Ultra-Low Attachment Spheroid Microplate, with Lid, Sterile (Corning, Cat.# 4515) or similar
- Optional: RNase A Solution (Cat.# A7973)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM477 available at: **www.promega.com/protocols** or for further information, please contact **techserv@promega.com**

Protocol:

1. Grow 3D microspheres in Corning® Ultra-Low Attachment 96 well plates or cells in Matrigel® Basement Membrane Matrix until DNA isolation is desired.
2. For spheroids: Use wide bore pipette tips to directly transfer spheroid in 100µl of media to well #1 of the Maxwell® RSC cartridge.
3. For cells embedded in Matrigel®: Use a wide bore pipette to transfer the Matrigel® to well #1 of the Maxwell® RSC cartridge.
4. Pipette more than 10 times to rinse pipet tip and thoroughly mix the lysis buffer and microtissues.
5. Prepare the deck tray following the technical manual TM477.
6. Add 100µl of Elution Buffer to the bottom of each elution tube.
7. Optional: Add 10µl RNase A to each culture prior to run and incubate at room temperature (25°C) for 10 minutes before adding to cartridge.
8. Run the Maxwell® RSC Tissue DNA method on the Maxwell® RSC Instrument.

Results:

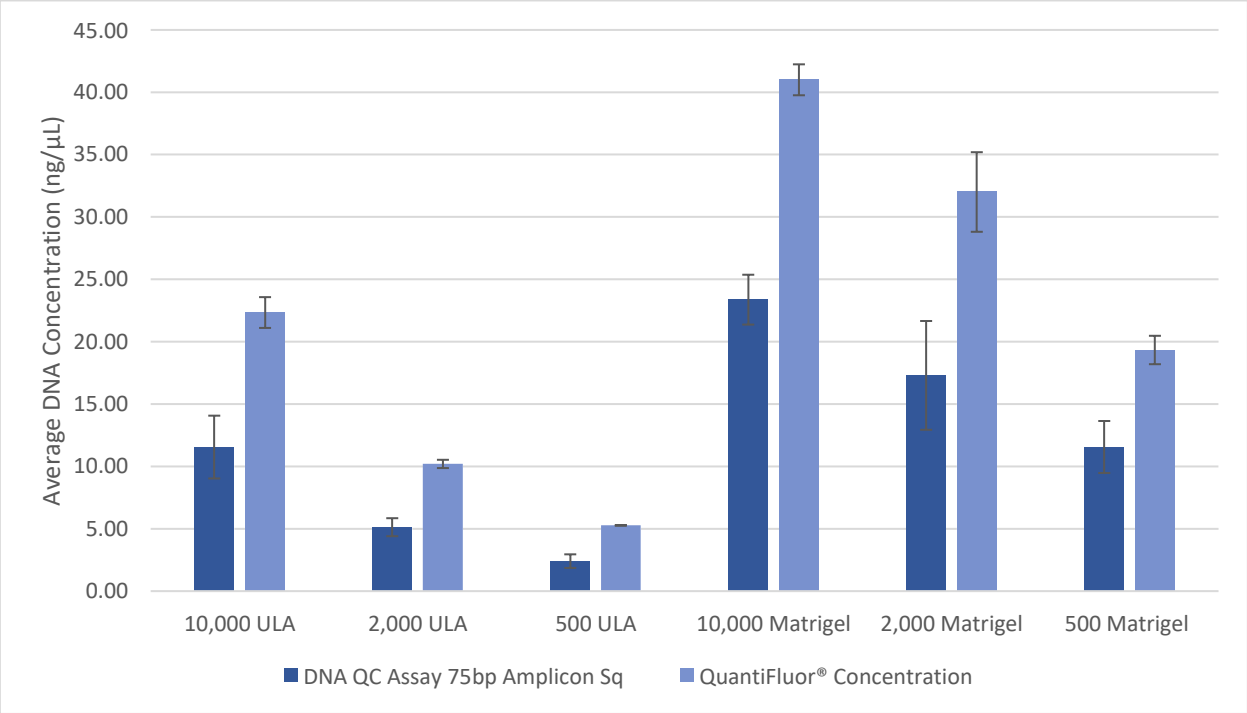

Figure 1. DNA isolation from HCT116 cells grown for four days in Corning® ULA plates or Corning® Matrigel® Basement Membrane Matrix using the Maxwell® RSC Cultured Cells DNA Kit (Cat.# AS1620) on the Maxwell® RSC Instrument (Cat.# AS4500). Along the x-axis the initial seeded cell number (10,000-500) and the growing condition (ULA plate or Matrigel®) are listed. The DNA concentration was measured using the Prototype DNA QC Assay and QuantiFluor® ONE dsDNA System (Cat.# E4871). The Prototype DNA QC Assay includes an internal positive control (IPC) and a shift in the Cq values would indicate possible inhibition. However no significant shift was observed (IPC shifts ≤ 0.18). The Prototype DNA QC Assay also allows for a proxy of DNA quality by comparing the ratio of small amplicons (75bp) to larger amplicons (300bp). No significant degradation was observed (Degradation ratio ≤ 1.5). Results are the average of N=3 per condition ± standard deviation.