Promega

Product Application

Automated DNA Isolation from 3D Microtissues

Isolate high quality, amplifiable DNA from 3D microtissues using the Maxwell® RSC Blood DNA Kit.

Kit: Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses: QuantiFluor® quantitation, gel electrophoresis

Sample Type(s): 3D microtissues

Materials Required:

- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Quantus™ Fluorometer (Cat.# E6150)
- QuantiFluor® ONE dsDNA System (Cat.# E4871)

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.

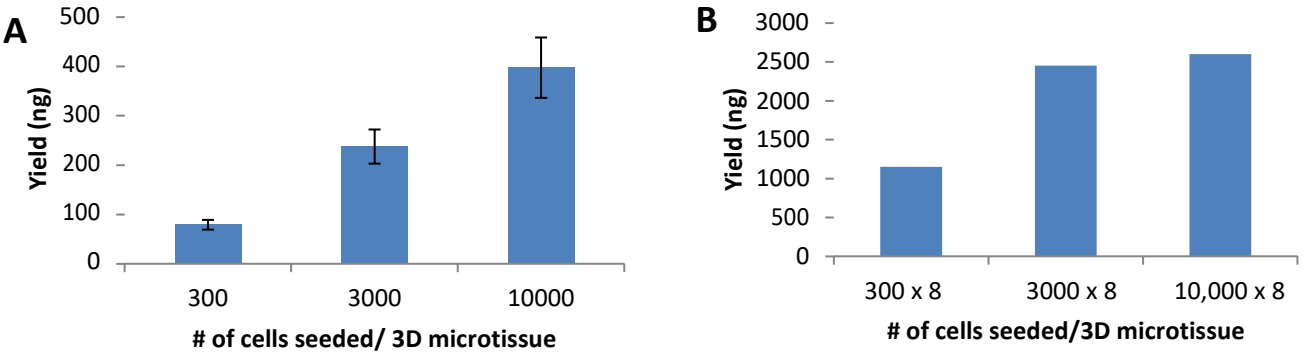
For further information, see Technical Manual TM419 available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Create microtissues and transfer to a 1.5ml microcentrifuge tube. In the following experiment, microtissues were created using the InSphero GravityPLUS™ Hanging Drop System (InSphero Cat.# CS06011).
2. Centrifuge the tube at 200 *x g* for 30 seconds and remove media by pipetting.
3. Add 300µl of 1X PBS and 30µl of Proteinase K (PK) to each sample.
4. Add 300µl of Lysis Buffer to each sample and vortex for 10 seconds.
5. Incubate this mixture at 56°C for 20 minutes.
6. Add the lysate to well #1 of the Maxwell® RSC cartridge.
7. Place plunger in well #8.
8. Add 50µl of Elution Buffer to the bottom of each Elution Tube and place into the deck tray.
9. Run the RSC Blood DNA method.

Results:
DNA was extracted from 3D microtissues (created using HCT116 cells and the InSphero GravityPLUS™ Hanging Drop System) of three different sizes using the protocol above.



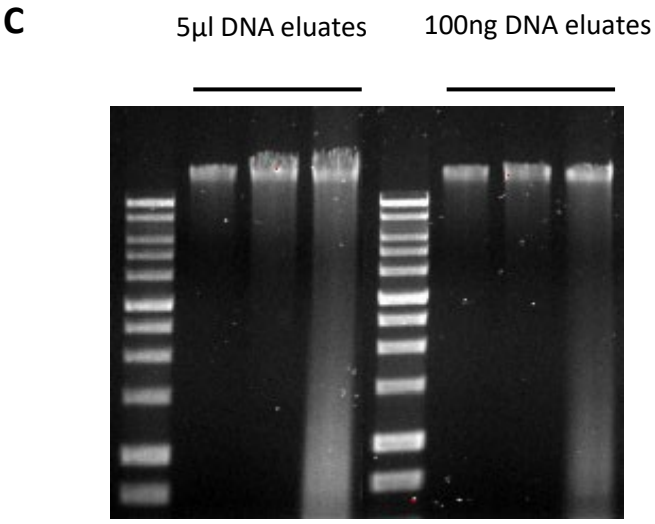


Figure 1. Analysis of DNA purified from 3D microtissues. A. DNA yields were determined using the Quantus™ Fluorometer (Cat.# E6150) and the QuantiFluor® ONE dsDNA System (Cat.# E2670). The graph illustrates that the amount of gDNA extracted increases with the size (volume) of each 3D microtissue. **B.** DNA yields were determined using the Quantus™ Fluorometer (Cat.# E6150) and the QuantiFluor® ONE dsDNA System (Cat.# E2670). This graph shows yields from 8 pooled microtissues of each size. **C.** Equal volume (5µl) and equal mass (100ng) of gDNA from all three sizes of microtissues were loaded onto a 1% agarose gel to assess quality (left to right: BenchTop 1kb DNA Ladder (Cat.# G7541), 300 cells, 3,000 cells and 10,000 cells seeded).