Promega

# Product Application

# Isolation of RNA from 3D microtissues after RealTime-Glo™ MT Cell Viability Assay

*Isolate high quality, amplifiable RNA from 3D microtissue after RealTime-Glo™ MT cell viability assay using the ReliaPrep™ RNA Tissue Miniprep kit.*

**Kit:** ReliaPrep™ RNA Tissue Miniprep kit (Cat. #Z6111)

**Analyses:** RealTime-Glo™ MT Cell Viability Assay, QuantiFluor® quantitation, Agilent 2100 Bioanalyzer

**Sample Type(s):** 3D microtissues

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

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manual #TM394, available at: **www.promega.com/protocols**

**Materials Required:**

- ReliaPrep™ RNA Tissue Miniprep Kit
- RealTime-Glo™ MT Cell Viability Assay, ( Cat. # G9711)
- Optional: Handheld Tissue homogenizer (Fisherbrand™ Pellet Pestle™ Cordless Motor)
- Microcentrifuge

**Protocol**:

Sample Preparation: (following the non-fibrous tissue protocol)

1. Prepare solutions as described in the technical manual (TM394).
2. Transferred each microtissue to a 1.5ml microfuge tube, centrifuged at 4000 *x g* for 2 minutes.
3. Removed medium and added 250µl LBA+TG buffer directly to the samples.
4. Vortexed samples for 10 seconds and pipeted up and down 10 times to lyse microtissues. To further lysis the microtissues a handheld homogenizer was used for 5-10 seconds.
5. Added 85µl of isopropanol to the mixture then vortexed for 5 sec.
6. Transfer lysate into a Reliaprep Minicolumn and centrifuge at 12,000-14,000 x *g* for 1 minute.
7. Discard the liquid from the Collection Tube. Add 500µl of RNA wash solution to the ReliaPrep™ Minicoulmn. Centrifuge 12,000-14,000 x *g* for 30 seconds.
8. Discard the liquid from the Collection Tube. Add 30µl of DNase I incubation mix directly to the column membrane. Incubate for 15 minutes at room temperature.
9. Add 200µl of Column Wash Solution. Centrifuge 12,000-14,000 x *g* for 15 seconds.
10. Add 500µl of RNA Wash Solution and centrifuge 12,000-14,000 x *g* for 30 seconds.
11. Place column into a new Collection Tube. Add 300µl of RNA Wash Solution and centrifuge at high speed for 2 minutes.

12. Place column into provided Elution Tube. Add 30µl of Nuclease-Free Water to the membrane. Centrifuge 12,000-14,000 x *g* for 1 minute. Remove the column and discard. Cap the Elution Tube containing the purified RNA and store at -70°C.
13. After the method was completed, placed eluates on ice.

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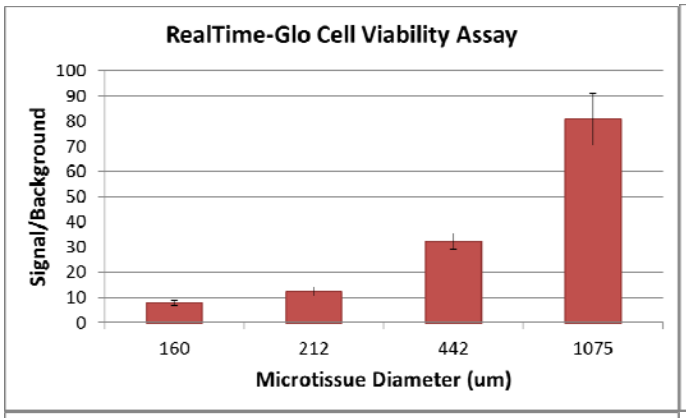
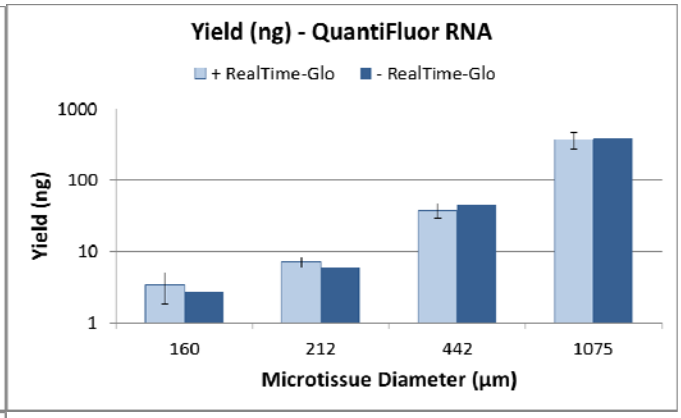
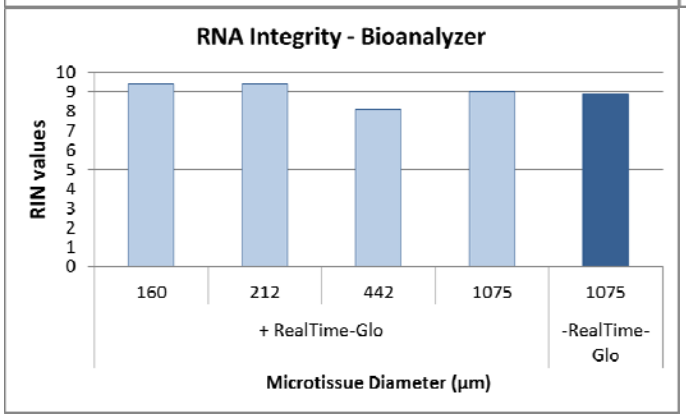
**Results: The above protocol was tested with four different sizes of HEK293 microtissues created using the Insphero GravityPLUS™ 3D Culture and Assay Platform (CS-06-001). Cell viability was tested using the RealTime-Glo™ MT Cell Viability Assay.**




**Upper Left:** Signal/Background values for 3D microtissues measured using the RealTime-Glo™ MT Cell Viability Assay (n=3). **Upper Right:** RNA yields as determined using the QuantiFluor® RNA system from 3D microtissue. **Lower Left:** RNA quality was determined using the Agilent bioanalyzer (RNA 6000 Pico Kit-5067-1513). RIN (RNA integrity numbers) range from 1-10, with 10 being the highest quality value.