Promega

Product Application

RNA isolation from human liver microtissues using the ReliaPrep™ RNA Tissue Miniprep System

Isolate high quality, amplifiable RNA from human liver microtissues using the ReliaPrep™ System.

Kit: ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6110)

Analyses: GoTaq® 1-Step RT-qPCR System, QuantiFluor® quantitation, Bioanalyzer

Sample Type(s): human liver microtissues

Input: 1-8 microtissues/RNA isolation

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:

- 3D InSight™ Human Liver Microtissues –Insphero - ~200um in diameter
- ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6110)
- Quantus™ Fluorometer (Cat. #E6150)
- QuantiFluor® RNA system (Cat. #E3310)
- Optional: Handheld Tissue homogenizer (Fisherbrand™ Pellet Pestle™ Cordless Motor)

Protocol:

Microtissue preparation

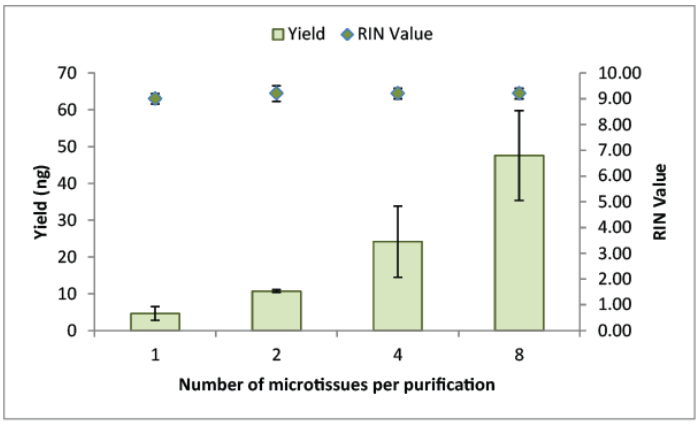
1. Wash microtissues with 50µl of 1x PBS.
2. Remove 25µl of microtissue(s) and PBS from the plate and place into a 1.5ml microfuge tube. Place desired number of microtissues per tube.
3. Spin at 4000 x g for 1min.
4. Remove PBS.
5. Continue directly to RNA isolation.

RNA isolation- ReliaPrep™ RNA Tissue Miniprep System

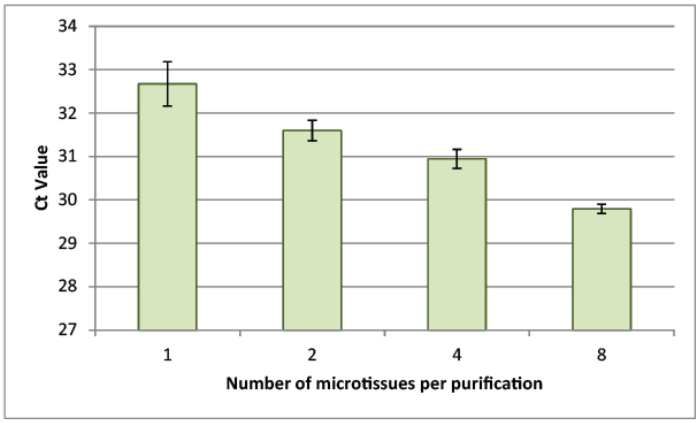
1. Add 250µl LBA+TG buffer directly to the samples.
2. Vortex samples for 10 seconds and pipet up and down 10 times to lyse microtissues. Optional: For more complete lysis: use a handheld homogenizer for 5-10 seconds.
3. Add 85µl of isopropanol to the mixture then vortex for 5 sec.
4. Follow TM394 protocol for all column binding and wash steps.
5. Elute RNAs in 30µl water.
6. After the method is complete, place eluates on ice.
7. Determine RNA concentrations and yields using the QuantiFluor® RNA system with the Quantus™ Fluorometer. For more details see TM396.

Results:

A.



B.



C.

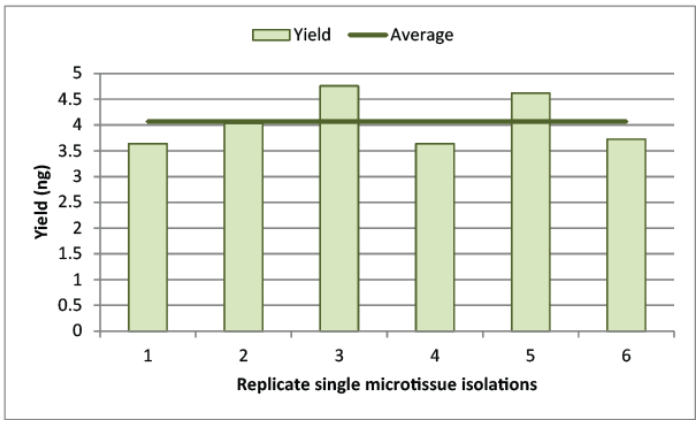


Figure: RNAs were isolated from human liver microtissues using the ReliaPrep™ RNA Tissue Miniprep System. **A –** RNAs were isolated from either 1, 2, 4, or 8 microtissues. Yields were determined using the QuantiFluor® RNA system with the Quantus™ Fluorometer. RNA integrity numbers (RIN) were determined using an Agilent Bioanalyzer and the RNA 6000 Pico kit. All values are greater than 9 indicating high quality RNA. **B –** HPRT specific primers were used with the GoTaq® 1-Step RT-qPCR System to show amplifiability of the isolated RNAs. **C –** RNAs were isolated from single microtissues to compare the average yield from one microtissue using this purification system. The line represents the average yield (n=6).