

## CELLINK SKIN RNA ISOLATION PROTOCOL

*This is a suggested procedure, please adjust according to your experimental needs.*

### Aim of the protocol:

This protocol is used for the isolation of RNA from cell-laden 3D bioprinted constructs to prepare for gene expression analysis by qPCR, next-generation sequencing or other RNA-based analysis methods.

### Materials needed:

- Liquid Nitrogen
- 2 mL reinforced reaction tubes (Sarstedt)
- $\beta$ -mercaptoethanol ( $\beta$ -ME or 2 M dithiothreitol (DTT))
- 70% and 96-100% ethanol
- 5-7 mm diameter stainless steel beads (Qiagen Cat No 69989/69990)
- TissueLyser LT (Qiagen Cat No 85600)
- RNeasy Plus MiniKit (Qiagen Cat No 74134)
- Cell-laden 3D bioprinted constructs

### Protocol:

Step n°	Title	Material	Description
1	Snap-freeze	▪ Liquid nitrogen	❖ Snap-freeze cell-laden 3D Bioprinted constructs in liquid nitrogen
2	Preparation for homogenization	▪ Reaction tubes ▪ Lysis buffer	❖ Transfer constructs to 2 mL reinforced reaction tubes containing one stainless steel bead and an appropriate volume of lysis buffer according to the RNeasy Plus MiniKit protocol (RLT-buffer from the MiniKit, supplemented with $\beta$ -ME or DTT)
3	Homogenization	▪ TissueLyser	❖ Homogenize the constructs using the TissueLyser for 10 min at 50 Hz
4	Add lysis buffer	▪ Lysis buffer	❖ Add lysis buffer for a total volume of 600 $\mu$ l
5	Centrifugation	▪ Centrifuge	❖ Centrifuge the constructs at 11,000 rpm for 1h at RT
6	RNA isolation	▪ RNeasy MiniKit	❖ Carefully remove the supernatant by pipetting and use it to purify RNA according to the steps in the RNeasy MiniKit Protocol.

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			<p>Roughly: Transfer the homogenized lysate to a gDNA Eliminator spin column, centrifuge and save the flow-through. Add 1 volume of 70% ethanol. Transfer the sample to an RNeasy spin column, centrifuge and discard the flow-through. Add Buffer RW1 to the RNeasy spin column, centrifuge and discard the flowthrough. Add Buffer RPE to the RNeasy spin column, centrifuge and discard the flow-through. Repeat. Place the RNeasy spin column in a collection tube. Add RNase-free water directly to the spin column membrane and centrifuge to elute the RNA.</p>
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Want to see our talented Biologist proceed to this protocol? Feel free to find the video here:

<https://www.youtube.com/...>

### Applications:

- ➔ Link to Videos of some applications
- ➔ photos of some applications



Want to see our existing tissue model?

Just go to <http://bioverse.co/> and discover a whole library of CAD files especially created for sharing 3D Bioprinting models.

### References:

Martínez H et al. 3D Bioprinting of Human Chondrocyte-laden Nanocellulose Hydrogels for Patient-specific Auricular Cartilage Regeneration. *Bioprinting*. 2016;1;22-35

- This protocol is optimized based on CELLINK™ Bioink, and may need further optimization for other bioinks. For more information, please contact: [bioinkteam@cellink.com](mailto:bioinkteam@cellink.com)

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