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Version:

RNA Isolation Protocol

Overview: 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:

- Liquid Nitrogen
- 2 mL reinforced reaction tubes (Sarstedt)
- β-mercaptoethanol (β-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:

1. Snap-freeze cell-laden 3D Bioprinted constructs in liquid nitrogen
2. 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 β-ME or DTT)
3. Homogenize the constructs using the TissueLyser for 10 min at 50 Hz
4. Add lysis buffer for a total volume of 600 µl
5. Centrifuge the constructs at 11,000 rpm for 1h at RT
6. Carefully remove the supernatant by pipetting and use it to purify RNA according to the steps in the RNeasy MiniKit Protocol.
    - 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 flow-though. 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.

Further Information:

This protocol is optimized based on CELLINK™ Bioink, and may need further optimization for other bioinks.

References:

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

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