

QGel™ Protocol

Gene expression analysis: how to extract mRNA from cells grown in QGel™ MT 3D Matrix

ABOUT THIS PROTOCOL

This is an example of how to extract cells from QGel™ MT 3D Matrix to enable gene expression analysis through RT-PCR or RT-qPCR. Further information of these protocols can be also found in published literature (e.g. Loessner D. et al, *Biomaterials* 2010). Note that this is only a guideline; volumes and concentrations of the different chemicals and incubation times require to be optimized depending on specific experimental condition.

PRODUCT SUPPORT

Brochures, FAQ and videos on: www.qgelbio.com/support

Suggested chemicals/solutions/kits:

- RNA extraction and purification kit (e.g. RNeasy® mini kit, Qiagen™)
- Standards material for RT-PCR or RT-qPCR procedure

Brief procedure description:

1. Transfer desired gel samples with encapsulated live cells in a new well plate (under cell culture hood) and wash them once with PBS for 5 minutes.
2. Disrupt the gel and homogenize the lysate either by using an homogenizer machine* or manually**.

Note: Freezing of the samples in liquid N₂ prior to homogenization, and subsequent storage at -80°C, is possible but optional.

3. Use RNeasy® kit, or other conventional methods for extraction of total mRNA, following instructions provided by the kit manufacturer (if using RNeasy®, start from the step involving the addition of 70% ethanol to the samples).
4. Extracted mRNA can be reverse transcribed and amplified following standard protocols for RT-PCR or RT-qPCR (e.g. Loessner D. et al, *Biomaterials* 2010).

Important note:

Depending on the quantity of cells per gel disc and their size, mRNA solutions extracted from different discs may need to be mixed in order to have enough mRNA for further amplification steps (i.e. ~ 1 µg).

*** Gel disruption and homogenization using an homogenizer:**

Transfer each samples in appropriate tubes (e.g. Lysing Matrix tube, MP Biomedicals) filled with 400µL Buffer RLT (from RNeasy® kit) and place the tubes into the homogenizer (e.g. FastPrep-24, MP Biomedicals). Homogenize twice for 40 sec. and centrifuge the samples for 3 minutes at 13000 rpm to separate RNA from insoluble debris. Transfer carefully the supernatants into new tubes and continue with step 3 above.

**** Manual mechanical gel disruption and homogenisation:**

Place the samples in conventional buffer (e.g. Buffer RLT from RNeasy® kit) used to lyse the cells and extract RNA. Grind the gels using a plastic pestle or minced by pipetting. Then, the lysate can be homogenized by passing through a 20-gauge (0.9mm) needle attached to a sterile plastic syringe. Centrifuge the samples for 3 minutes at 13000 rpm to separate RNA from insoluble debris. Transfer carefully the supernatants into new tubes and continue with step 3 above.