1. Objective

This protocol describes the procedure for RNA isolation from cells cultured in the OrganoPlate® using QIAGEN's RNeasy® Micro kit.

2. Background

The OrganoPlate® allows the culture of in-gel tissues (e.g. neuronal networks or liver cells), the culture of tubular tissues (e.g. endothelial or epithelial barriers), or combinations of both. Cultures can be lysed by perfusing a buffer through the channels of the microfluidic chips. RNA is extracted from the lysate using the RNeasy® Micro kit. The extracted RNA can be used for cDNA synthesis and qPCR analysis.

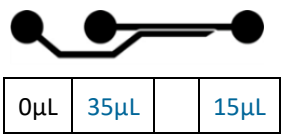
3. Materials

- OrganoPlate® 2-lane or 3-lane (MIMETAS, 9605-400-B or 4004-400-B) with cultured cells
- RNeasy® Micro Kit (QIAGEN, cat# 74004)
- 1.5 mL Eppendorf centrifugation tubes

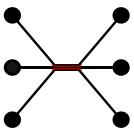
4. Procedure

Lysis

1. Aspirate medium from all wells of the chips you want to lyse
2. Add QIAGEN lysis buffer to chips
    a. Adjust the volume depending on the type of plate you are using (i.e. OrganoPlate® 2-lane or 3-lane) and the location of the cells that you want to lyse inside the chips (i.e. tubular structure in top channel only or complex co-culture with cells in all channels)
    b. The pipetting schemes below show several options that can be used

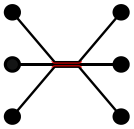


0µL	35µL		15µL

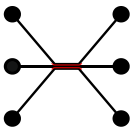


35µL		15µL
0µL		0µL
0µL		0µL

10µL	30µL		10µL



30µL		10µL
0µL		0µL
10µL		10µL



20µL		10µL
5µL		5µL
5µL		5µL

3. Incubate the lysis buffer for 30-60 s or until the culture is fully lysed (check under the microscope)
4. Collect the lysate from the chips you want to pool (see section "**tips & troubleshooting**") into one sample in an RNase-free Eppendorf tube
5. Add lysis buffer to the Eppendorf tube to reach a final volume of 350 µL/sample
6. Store samples at -80°C or continue with RNA isolation

RNA isolation

Perform all steps described in the manufacturer's protocol for the RNeasy® Micro Kit.

5. Tips & troubleshooting

Obtaining sufficient RNA yields

- For most applications, pooling the lysate of several chips into one sample is required
- For example, when isolating RNA from tubular cultures, we recommend pooling the lysate of 3-5 chips into one sample. When using the RNeasy® Micro kit, use 50 µL of lysis buffer per chip. Collect the lysates of 2-5 chips in an Eppendorf tube and add lysis buffer to reach a final volume of 350 µL. Use the obtained sample for further RNA isolation using the RNeasy® Mini Kit.
- In-gel cultures often result in lower RNA yields per chip, due to lower cell numbers compared to tubular cultures. Pooling a higher number of chips may be necessary to obtain sufficient RNA.
- In case insufficient yields are obtained, try the classic TRIzol® RNA extraction method. Pool several chips and follow the procedure described in the TRIzol® manufacturer's protocol and use glycogen as a carrier. This procedure generally results in higher yields.
    - TRIzol® lyses cultures very quickly (within 1-2 minutes). Remove the lysate as soon as the culture is lysed. Do not leave TRIzol® in the OrganoPlate® for longer than 5 minutes
    - Discard the OrganoPlate® after usage of TRIzol®

MIMETAS product list

Cat. No.	Product Name
MI-AR-CC-01	OrganoReady® Caco-2
9605-400-B	OrganoPlate® 2-lane
4004-400-B	OrganoPlate® 3-lane 40
6405-400-B	OrganoPlate® 3-lane 64
6401-400-B	OrganoPlate® Graft
MI-OFPR-S	OrganoFlow® S
MI-OFPR-L	OrganoFlow® L
MI-OT-1	OrganoTEER®

Contact information

For questions, please contact us through the e-mail addresses stated below
Purchasing: order@mimetas.com
Customer service: info@mimetas.com
Technical support: support@mimetas.com

MIMETAS Europe
J.H. Oortweg 19
2333 CH, Leiden
The Netherlands
Phone: +31 (0)85 888 3161

MIMETAS USA
704 Quince Orchard Road
Suite 260, MD 20878
Gaithersburg, USA
+1 (833) 646-3827

MIMETAS Japan
4F Tekko Building,
1-8-2 Marunouchi, Chiyoda-Ku
Tokyo, 100-0005, Japan
+81 3-6870-7235