RNA isolation from OrganoPlate® cultures

1. Objective

This protocol describes the procedure for RNA isolation from cells cultured in the OrganoPlate® using the 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 lysis 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 qPCR and RNA sequencing analysis.

3. Materials

- OrganoPlate® 2-lane or 3-lane (MIMETAS, 9605-400-B, 4004-400-B, or 6405-400-B) with cultured cells
- RNeasy® Micro Kit (QIAGEN, cat# 74004)
- 1.5 mL Eppendorf centrifugation tubes

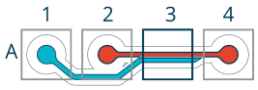
4. Procedure

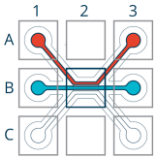
- For most applications, pooling the lysate of several chips into one sample is required.
- For example, when isolating RNA from tubular cultures, we recommend pooling the lysate of 3-5 chips into one sample. When using the RNeasy® Micro kit, use 50 µL of lysis buffer per chip. Collect the lysates of 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.

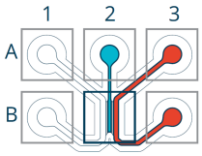
Lysis

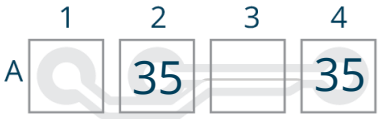
1. Aspirate medium from all channels of the chips you want to lyse.
2. Add QIAGEN lysis buffer to the chips as:
    a. Adjust the volume depending on the type of plate you are using (i.e. OrganoPlate® 2-lane or 3-lane) and the location of the cells that you want to lyse inside the chips (i.e. tubular structure in top perfusion channel or right perfusion channel only or complex co-culture with cells in all channels)
    NOTE: for tubular structures, it is important to have a difference in volumes from the inlet to the outlet to induce flow through the channel. For example, in 3 lane-40 most tubes are in the top perfusion channel, therefore the top inlet has the largest volume. For 3-lane 64, if the tubular structure is in the right perfusion channel, the right inlet should have the largest volume.
    b. The pipetting schemes on the next page show several options that can be used (but are not the only options available)

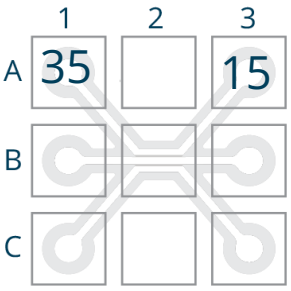
Lysis of cultures in one perfusion channel
Cell tubules in red, grown against an ECM gel in blue

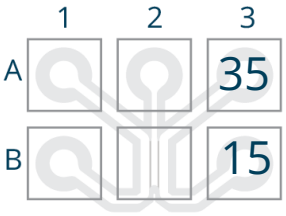






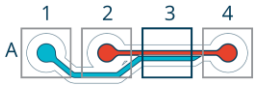


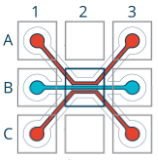


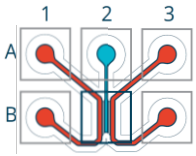


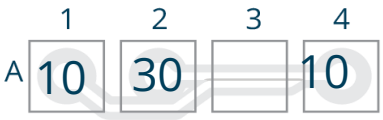
Lysis of cultures in two channels
Cell tubule in red, grown against an in-gel cell culture in blue

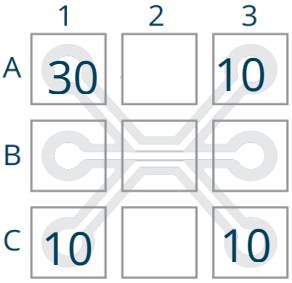
Lysis of cell cultures in two channels
Tubules or in gel cultures in red grown against ECM in blue

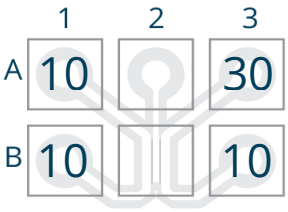




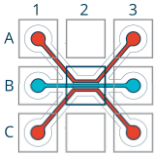


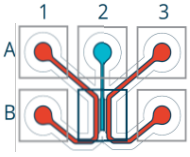


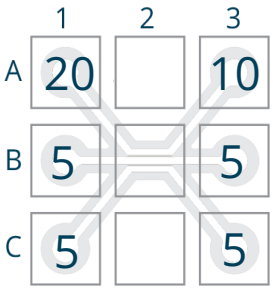


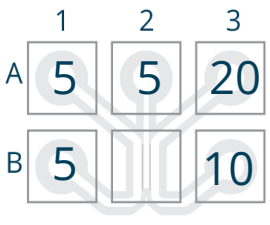


Lysis of cell cultures in three channels
Two tubules and one in- gel culture









3. Incubate the lysis buffer for 30-60 s or until the culture is fully lysed (check under a wide field light 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
   Store samples at -80°C or continue with RNA isolation

RNA isolation

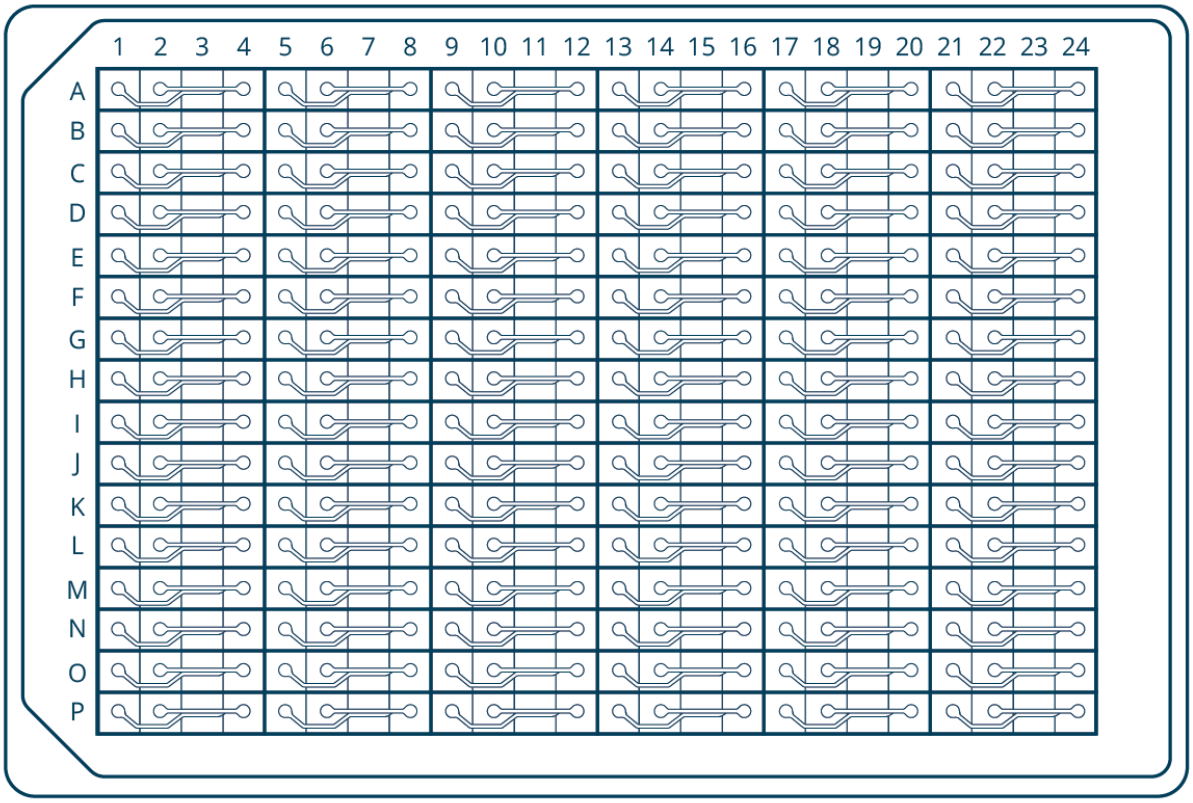
Perform all steps described in the manufacturer's protocol for the RNeasy® Micro Kit.

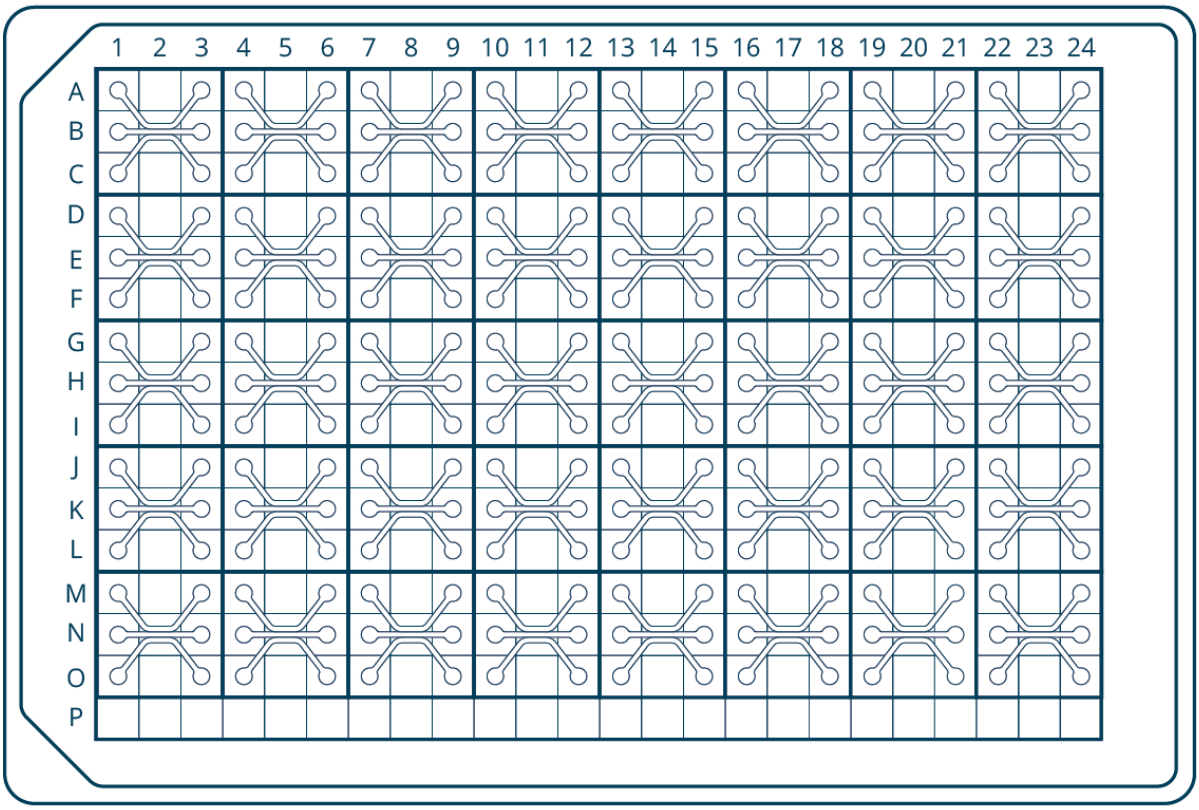
5. Troubleshooting

Obtaining sufficient RNA yields

- 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, either increase the number of pooled replicates or 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®

Plates layout

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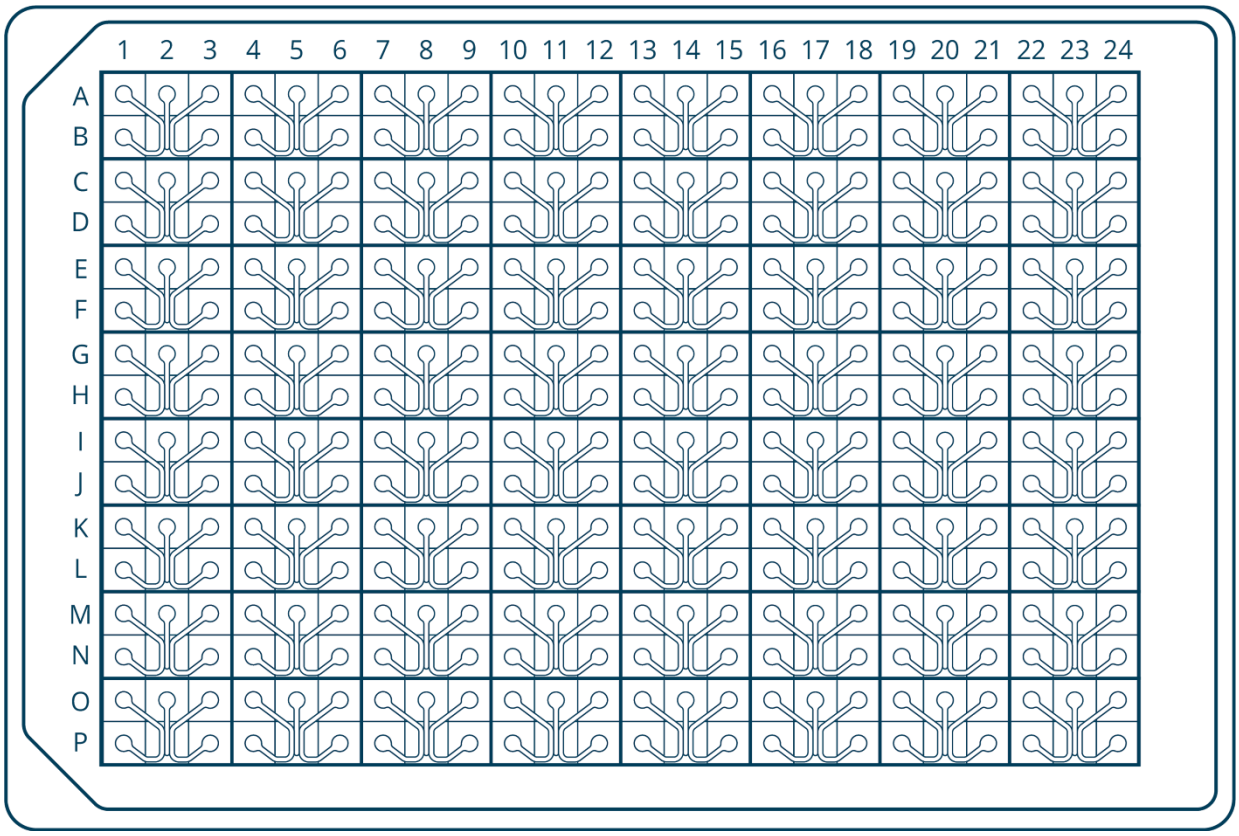
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MIMETAS Product List

Cat. No.	Product Name
MI-OR-CC-01	OrganoReady® Colon Caco-2 3-lane 40
MI-OR-CC-02	OrganoReady® Colon Caco-2 3-lane 64
MI-OR-BV-01	OrganoReady® Blood Vessel HUVEC 3-lane 40
MI-OR-BV-02	OrganoReady® Blood Vessel HUVEC 3-lane 64
MI-OR-AN-01	OrganoReady® Angiogenesis HUVEC 3-lane 64
MI-OR-HB-01	OrganoReady® BBB HBMEC 3-lane 40
MI-OR-HB-02	OrganoReady® BBB HBMEC 3-lane 64
MI-OR-VB-01	OrganoReady® Vascular Bed HUVEC
MI-OR-CO-CU -01	OrganoReady® Collagen 3-lane 40
MI-OR-CO-CU-02	OrganoReady® Collagen 3-lane 64
9605-400-B	OrganoPlate® 2-lane 96
4004-400-B	OrganoPlate®3-lane 40
6405-400-B	OrganoPlate®3-lane 64
6401-400-B	OrganoPlate® Graft
MI-OFPR-S	OrganoPlate® S
MI-OFPR-L	OrganoPlate® L
MI-OT-VP2	OrganoPlate® Standard package

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