RNA extraction protocols

The RNA extraction of cells cultured in BIOMIMESYS® is performed according to the manufacturer's instructions of commercial mRNA extraction kits.

BIOMIMESYS® is compatible with manual (Trizol) and commercially available mRNA extraction kits such as QIAGEN *RNeasy Micro* or *Macherey-Nagel NucleoSpin RNA XS.*

Below are recommendations to optimise RNA extraction:

- *NucleoSpin RNA XS kit from Macherey-Nagel* :

- Lyse cells by vortexing hydrogels thoroughly 2 x 5 seconds in **100µL buffer RA1 + 2µL TCEP** (for 2 hydrogels).
- Directly place the 2 hydrogels, side by side, in the purple clarification column, then place iton a collecting tube.
- Centrifuge 5 minutes at 10 000g.
- Measure the recovered filtrate volume with a pipette and add the same volume of 70% ethanol. Mix by pipetting (at least 5 times) before dispensing the mixture onto the blue column and continue RNA extraction according to the manufacturer instructions (step 6).

- *RNeasy Micro Kit from Qiagen* :

- Lyse cells by vortexing hydrogels thoroughly 2 x 5 seconds in **350µL buffer RLT** (for 2 hydrogels).
- Break down hydrogels in lysis buffer and by aspirating total volume in 1mL syringe with a 1.9mm needle.
- Centrifuge 5 minutes at 10 000g, collect supernatant.
- Measure the collected supernatant volume, add same volume of 70% ethanol and mix well by pipetting several times.
- Transfer the mixture onto the RNeasy Min Elute spin (pink column) and continue RNA extraction according to the manufacturer's instructions (step 3 from Quick Start Protocol).

analytikjena • *InnuPrep RNA Mini from AnalytikJena* :

- Lyse cells by vortexing hydrogels thoroughly 2 x 5 seconds in **400µL buffer RL** (for 2 hydrogels).
- Directly place the 2 hydrogels, side by side, in the blue Spin Filter D column, and place it on a collecting tube.
- Centrifuge 5 minutes at 10 000g.
- Measure the filtrate volume, add same volume of 70% ethanol and mix well by pipetting several times.
- Transfer the mix onto the Spin Filter R column (purple column) and continue RNA extraction according to the manufacturer's instructions (step 4 RNA extraction from eucaryotic cells protocol).

- Using *Trizol/Chloroform/Isopropanol extraction* :
  - Lyse cells by vortexing hydrogels thoroughly 2 x 5 seconds in 1ml Trizol (for 1 to 5 hydrogels).
  - Break down/destroy hydrogels in Trizol by aspirating total volume in 1mL syringe with 1.9mm needle.

    Optional step: successive freeze-drying of lysates containing hydrogels.
  - Add 200µL of Chloroform, vortex 2 x 5 seconds and incubate 3 minutes at room temperature.
  - Centrifuge 20 minutes at 10 000g at 4°C.
  - Collect aqueous phase in a new tube.
  - Add 500µL of Isopropanol, vortex 2 x 5 seconds and incubate 20 minutes at room temperature or overnight at -20°C.
  - Centrifuge 20 minutes at 10 000g at 4°C.
  - Discard supernatant without aspirating the pellet, add 1 mL of 75% ethanol and vortex 2 x 5 seconds.
  - Centrifuge 15 minutes at 10 000g at 4°C.
  - Remove supernatant without aspirating the pellet, add 1 mL of 75% ethanol, vortex 2 x 5 seconds, and centrifuge 15 minutes at 10 000g at 4°C.

- Vacuum or air dry the RNA pellet.
- Resuspend the RNA pellet in 10-20µL RNase-free water by passing the solution up and down several times through a pipette tip.
- Incubate in a water bath or heat block set at 55°C for 5 minutes.
- Purify obtained RNA or proceed to downstream applications.

Yield per hydrogel of a 96 well plate, according to kits used :

Kits	Cells	Culture time	Yield/hydrogel	Ratio (OD 260/280nm)
NucleoSpin RNA XS Macherey-Nagel	HT-29	7 days	1-2 µg	2.09-2.12
		14 days	1.5-3 µg	
		30 days	8 µg	
	HepG2	30 days	600 ng	2.09-2.08
	3T3-L1	8 days	840 ng	2.14-2.18
innuPrep RNA Mini Eurobio	HT-29	30 days	8 µg	2.09-2.13
	HepG2	30 days	400 ng	
RNeasy Micro Kit Qiagen	3T3-L1	8 days	940 ng	2.08-2.09
Trizol/ Chloroform/ Isopropanol	HT-29	30 days	14-23 µg	1.83-1.94
	HWP	6 days	681 ng	1,83-2,13
		12 days	411 ng	
		19 days	417 ng	
		26 days	593 ng	
		31 days	491 ng	