

The RNA extraction of cells cultured in BIOMIMESYS®

The RNA extraction of cells cultured in BIOMIMESYS® should be 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

1. NucleoSpin RNA XS kit from Macherey-Nagel

- Lyse cells by vortexing hydroscallops thoroughly 2 x 5 seconds in 100µL buffer RA1 + 2µL TCEP (for 2 hydroscallops).
- Directly place the 2 hydroscallops, side by side, in the purple clarification column, then place it on 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).



2. RNeasy Micro Kit from Qiagen

- Lyse cells by vortexing hydroscallops thoroughly 2 x 5 seconds in 350µL buffer RLT (for 2 hydroscallops).
- Break down hydroscallops 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).



3. InnuPrep RNA Mini from AnalytikJena

- Lyse cells by vortexing hydroscallops thoroughly 2 x 5 seconds in **analytikjena** 400µL buffer RL (for 2 hydroscallops).
- Directly place the 2 hydroscallops, 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).

4. Using Trizol/Chloroform/Isopropanol extraction

- Lyse cells by vortexing hydroscallops thoroughly 2 x 5 seconds in 1ml Trizol (for 1 to 5 hydroscallops).
- Break down/destroy hydroscallops in Trizol by aspirating total volume in 1mL syringe with 1.9mm needle. Optional step: successive freeze-drying of lysates containing hydroscallops.
- 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.

5. Yield per hydroscaffold of a 96-well plate, according to kits used

Kits	Cells	Culture time	Yield/hydroscaffold	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	

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