Total RNA isolation with RNeasy MICRO (Qiagen) SPIN Protocol

RNeasy Micro Kit (50) Ref:74004
QIAShredder (250) Ref:79656

High quality of RNA is then eluted in 30ul, or more, of water. P10
Useful for 1(?) up to 0.5x10^6 cells. Samples maximum 0.5x10^6 cells using the spin protocol.
For small number of cells homogenisation can be done by vortexing. P23
The binding capacity of the column is 45ug RNA. P39
A minimum number of 100 cells can generally be processed with RNeasy mini columns. P30
All centrifugation must be performed at 20-25°C.

- Resuspend dry pellet in 350ul of ***RLT buffer***. Ensure that β-ME has been added to the RLT buffer less than a month before (add 10ul β-ME per 1ml of RLT buffer. d=1/100).
Homogenize the samples by pipetting up and down and Vortex several times.
If < 1x10^5 cells are processed, the cells can be homogenized by vortexing. Make sure no clumps cells are left.

- When processing < 5000 cells add 20ng of ***carrier RNA*** to the 350ul of RLT buffer. Stock solution frozen at 400ug/ml. Dilute it to 4ug/ml (4ng/ul) and add 5ul to the 350ul of RLT buffer.

- Transfer the 350ul lysis solution onto a **QIAShredder spin column (purple)** placed in a 2ml collection tube. Centrifuge for **2min** at MAX speed.

- Add 1Vol (= 350µl) of ***Ehanol 70%*** to the homogenized lysate and mix well by pipetting.

- Transfer the 700ul of the sample into an **RNeasy MinElute spin (pink)** column placed in a 2ml collection tube. Close the tube and centrifuge for **15sec** at 8000g (10000rpm). Discard flow through.

- Add ***350ul RW1*** buffer into the column. Close the tube and centrifuge for **15sec** at 8000g (10000rpm). Discard flow through.

- Dilute 10ul of (RNAse-free) DNaseI stock solution to 70ul RDD buffer and mix by pipetting.
Add the 80ul of DNaseI solution to the column and incubate 15min at RT.

- Wash with 350ul ***RW1 buffer*** into the column. Close the tube and centrifuge for **15sec** at 8000g (10000rpm). Discard flow through and collection tube.

- Transfer the RNeasy MinElute spin column into a new 2ml collection tube. Add 500ul ***RPE buffer*** into the column. Ensure that ethanol absolute has been added to the RPE buffer before (add 220ml ethanol absolute per 55ml of RPE buffer).
Close the tube and centrifuge for **15sec** at 8000g (10000rpm). Discard flow through.

- Add 500ul of ***Ehanol 80%*** into the RNeasy MinElute spin column. Close the tube and centrifuge for **2min** at 8000g (10000rpm) to dry RNeasy silica gel membrane. Discard flow through and collection tube.

- Transfer the RNeasy MinElute spin column into a new 2ml collection tube. Open the cap of the spin column and centrifugate for **5min** at MAX speed.

- To elute, transfer RNeasy columns to a new 1.5ml eppendorf tube (cut top and numbered). Add 22ul *(can be reduce to 14ul)* ***RNase free water*** directly to the RNeasy silica gel membrane.
Centrifuge for **1min** at MAX speed.

- Transfer the 20ul containing RNA in new small eppendorf tubes, which contain 2ul of ***oligo-dN$_6$*** (3mg/ml) → 2µl/sample. (Promega: Random Primers 20ug, Ref: C118A, £28.98).

Denature 5-10 min @ 70°C.
Shock cool on ice

Prepare the mix for the reverse transcription.

Reverse trancription:

Random primers used : Promega Random Primers 20ug IBR-C1181, 1*20ug, £28.98
Add RT-Mix containing → 18µl/sample

Per sample	20ul +	2ul +	18ul =	40ul
	RNA	dN6	RT-Mix	

6.375µl H_2O
+ 8µl 5X first strand buffer (Promega Ref IBR-M1701)
+ 1.5µl dNTP (10mM) (Invitrogen: dNTP Set 100 mM 4 X 25umol, Ref IBR10297018, £89.10)
+ 0.625µl RNAse Inhibitor 25 Units (Invitrogen, RNaseOUT™ Recombinant Ribonuclease Inhibitor IBR10777019, 40Units/ul, £62.65)
+ 1.5µl M-MLV 300Units (Promega, M-MLV Reverse Transcriptase, 200 U/ul Ref IBR-M1701)

Mix well using pipette

1h 42C (41C, block control and heated lid in Hybaid PCR machine)
Heat 10' to 90C to inactivate RT
Dilute to 100 µl with TE-buffer if necessary
Store at 4C (-20C for longer periods)