Aug 17, 2020

Cell harvest and RNA prep using Qiagen RNAeasy Kit

Katrin Blondrath[1]
[1]RIKEN

1 Works for me This protocol is published without a DOI.

Katrin Blondrath

PROTOCOL CITATION

Katrin Blondrath 2020. Cell harvest and RNA prep using Qiagen RNAeasy Kit. **protocols.io** https://protocols.io/view/cell-harvest-and-rna-prep-using-qiagen-rnaeasy-kit-bf4sjqwe

CREATED

May 07, 2020

LAST MODIFIED

Aug 17, 2020

PROTOCOL INTEGER ID

36722

MATERIALS

NAME	CATALOG #	VENDOR
RNAeasy mini kit	74106	Qiagen
Parafilm		
beta mercaptoethanol		
low bind nuclease free eppendorf tubes		
RNAse zap		
Ice		
Ethanol		
Nano drop or similar		
384well plate		
seal for 384well plate		
One-step PrimeScript RT-PCR kit for real-time RT-PCR		
One Step TB Green™ PrimeScript™ RT-PCR Kit II	RR086A	Takara

BEFORE STARTING

have ice ready
wipe surfaces and pipettes with RNAse zap
prepare 70% ethanol

Cell harvesting and RNA extraction

1 Aspirate medium from 6 well plates, wash once with PBS

2 Add **500 µl** lysis buffer containig freshly added beta-mercaptoethanol to each well (lysis buffer provided in RNAeasy kit)

Beta-mercaptoethanol should be added freshly every time performing the experiment

3 (OPTIONAL) Wrap plates in parafilm, store at **-80 °C** until extraction

4 (Defrost plates and) Scrap with 1000 ul pipette tip and pipett up and down the well, transfer into low bind nuclease-free tube

5 Extract according to manufacturer's protocol using Qiagen RNAeasy kit (including DNAse treatment step) 1h

Storage

6 Measure RNA concentration using Nanodrop and store at **-80 °C** or continue with qPCR

qPCR

7 Dilute samples to have equal concentrations of 2500 ng of each sample.
Use **2 µl** of this diluted RNA directly in 10 ul reaction to achieve 50nM per well of 384 well reaction plate

8 Mix contents of One Step TB Green™ PrimeScript™ RT-PCR Kit II (in case running a whole 384well plate)

Pre Master: Mix contents of entire kit:

- **2500 µl** TB Green buffer
- **200 µl** RT/Polymerage enzyme
- **100 µl** ROX
- **800 µl** H_2O
- **3600 µl total**

Adjust accordingly to the total number of required reactions

1x Mastermix:

		1x in micro-liters
ENZYME		0.4
TB green Buffer 4		5
Primer 30uM F/R mix		0.8
RNA		2
ROX		0.4
H2O		1.8

9 **Final master mix:**

Per reaction/ per well of 384well plate = 10 µl

Multiply by amount of samples and add ~20% dead volume.