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1 Step RT- QPCR

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We use this protocol and it's working

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Abstract

description of how to do a 1 step rt qpcr

Organoid RNA extraction

1 All RNA was extracted using the following protocol as described in our paper dx.doi.org/10.17504/protocols.io.81wgbr95qlpk/v1

1 Step RT-QPCR

2
- Use RNase-free technique throughout.
- Decide your multiplex groups (up to 4 targets per well, including GAPDH), making sure fluorophores do not overlap.
- Thaw master mix, probes, and RNA on ice. Mix gently and quick-spin.

3 Measure RNA concentration for each sample.

4 Dilute each RNA sample with nuclease-free water so all samples have the same concentration (so that 2 µL gives the same RNA input for every sample)

5 Keep normalized RNA on ice

6 Create a plate map:
List each sample.
For each probe set/condition, assign 2 technical replicates per sample.
Total wells = samples × conditions × replicate

7 Label your 384-well plate orientation and corresponding wells

8 For each multiplex condition (each “probe set”), make a probe mix so pipetting is consistent.In a labeled tube for Probe Set 1, combine the probes for that multiplex (up to 4 probes).Repeat for Probe Set 2, 3, and 4

9 Make one “reaction mix” per probe set that contains everything except RNA. Per well (10 µL total):
- 5.0 µL IDT PrimeTime One-Step RT-qPCR Master Mix
- 0.5 µL of each probe (up to 4 probes total; 2.0 µL max probe volume)
- Nuclease-free water to bring the non-RNA volume to 8.0 µL
- RNA will be 2.0 µL added later

10 For each probe set, calculate number of reactions needed:
(number of wells for that probe set) × (1.1 or 1.2) to account for pipetting loss

11 In a chilled tube, combine for that probe set:
PrimeTime One-Step RT-qPCR Master Mix (5 µL × number of reactions)
Probe mix components (0.5 µL each probe × number of reactions)
Nuclease-free water to reach 8 µL per reaction

12 Mix gently by pipetting, then quick-spin.
Keep mixes on ice and protected from light.

13 Add 8.0 µL of the appropriate probe-set reaction mix into each assigned well of the 384-well plate.
Add 2.0 µL of the corresponding normalized RNA sample into each well.

14 For no-template controls (NTCs), add 2.0 µL nuclease-free water instead of RNA.
Seal the plate with an optical seal. Quick-spin the plate (brief spin) to remove bubbles and collect liquid at the bottom.

15 Load the plate into the Bio-Rad CFX Opus 384.
Set up the run as a one-step RT-qPCR multiplex assay:
- Assign fluorophores/channels matching your probes.
- Confirm plate type (384-well) and reaction volume (10 µL).

Use the cycling program recommended by IDT for PrimeTime One-Step RT-qPCR Master Mix
Start the run.

Analysis

16 After the run, verify amplification curves and baseline/threshold settings

17 Confirm NTCs show no amplification.

18 Export Cq values and analyze using your preferred method