Quick-Start Protocol May 2018

QIAseq™ UPX 3' Targeted RNA Panel

Part 1: Cell lysis, reverse transcription

Upon receipt, store the QIAseq UPX 3' Targeted RNA Panel at –30 to –15°C. The QIAseq Beads and Cell Index (ID) RT Plate should be stored at 4°C.

Further information

- *QIAseq UPX 3' Targeted RNA Library Handbook*: **www.qiagen.com/HB-2480**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- If using the multi-use 96-well Cell ID RT Plate, consult the handbook.
- The recommended starting amount is 1–100 cells.
- For single cells, a minimum of 24 cells must be multiplexed per sample index.
- If purified RNA is used, consult the handbook.
- Ensure reactions are thoroughly mixed (12 times), prepared and incubated at recommended temperatures. Do not vortex.

Cell lysis

1. Prepare the Cell Lysis Premix on ice as described in Table 1.

Table 1 Preparation of Cell Lysis Premix

Component	Volume 8 wells	Volume 24 wells	Volume 96 wells	Volume 384 wells
Cell Lysis Buffer	10 µl	30 µl	120 µl	480 µl
RNase Inhibitor	2.5 µl	7.5 µl	30 µl	120 µl
Nuclease-free Water	17.5 µl	52.5 µl	210 µl	840 µl
Total volume	**30 µl**	**90 µl**	**360 µl**	**1440 µl**

2. Aliquot 3 µl of the premix into the required wells of the single-use Cell ID RT Plate.
3. Capture cells into the plate containing the Cell Lysis Premix.
4. Incubate for 15 min on ice.
5. Freeze at –80°C or proceed immediately with "Reverse transcription".

Reverse transcription

6. Prepare the RT Premix on ice as described in Table 2.

Table 2 Preparation of RT Premix for single-use UPX 3' Cell ID RT Plates

Component	Volume 8 wells	Volume 24 wells	Volume 96 wells	Volume 384 wells
3' Tar RT Buffer	10 µl	30 µl	120 µl	480 µl
EZ Reverse Transcriptase	2.5 µl	7.5 µl	30 µl	120 µl
Nuclease-free Water	5 µl	15 µl	60 µl	240 µl
ERCC diluted 1.25 x 10^6-fold* or Nuclease-free Water	2.5 µl	7.5 µl	30 µl	120 µl
Total volume	**20 µl**	**60 µl**	**240 µl**	**960 µl**

*For single-cell analysis, ERCC can be added as a control. Prepare the ERCC by diluting the stock 1.25x10^6—fold using 0.1X TE Buffer.

7. Aliquot 2 µl of the RT Premix into the wells.
8. Incubate as follows: 5 min at 25°C, 1 h at 42°C, 5 min at 95°C, and hold at 4°C.
9. Combine the synthesized cDNA from the different wells into one, 2 ml microcentrifuge tube. Up to 96 wells can be combined in one tube.
10. Add 1.3X (volume) QIAseq Beads to the combined cDNA synthesis reactions as described in Table 3. Mix well by pipetting up and down 12 times.

Table 3 Addition of QIAseq Beads for cDNA cleanup

Number of wells combined	QIAseq Bead volume
8	52 µl
24	156 µl
96*	624 µl

* When working with 384 wells, perform the cleanup as 4 sets of 96 wells. Combine supernatants at step 18.

11. Incubate for 5 min at room temperature.
12. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.
13. With the beads still on the magnetic stand, add 200 µl 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.

14. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.
15. With the tube (caps opened) still on the magnetic stand, air dry for 10 min.

    Note: Visually inspect that the pellet is completely dry.
16. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 52 µl nuclease-free water. Mix well by pipetting.
17. Return the tube/plate to the magnetic rack until the solution has cleared.
18. Transfer 50 µl of the supernatant to clean tubes/plate.

    Important: With 384 wells, mixture can be combined across all four sets to consolidate all 384 indices into one tube.
19. Add 1.3X volume QIAseq Beads to the supernatant. Mix well by pipetting up and down 12 times.

    Note: QIAseq Bead volume will be 65 µl if supernatant is 50 µl.
20. Incubate for 5 min at room temperature.
21. Place the tubes on a magnetic rack for 5 min. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.
22. With the beads still on the magnetic stand, add 200 µl 80% ethanol. Rotate the tube (3 times) to wash the beads. Carefully remove and discard the wash.
23. Repeat the ethanol wash. Completely remove all traces of the ethanol wash.
24. With the tube (caps opened) still on the magnetic stand, air dry for 10 min.

    Note: Visually inspect that the pellet is completely dry.
25. Remove the beads from the magnetic stand, and elute the DNA from the beads by adding 12 µl nuclease-free water. Mix well by pipetting.
26. Return the tube/plate to the magnetic rack until the solution has cleared.
27. Transfer 10 µl of the supernatant to clean tubes/plate.

    Note: From this point of the protocol, the procedures assume that all cDNA wells (8, 24, 96 or 384) have been combined into a single tube.
28. Proceed with "Single primer extension" in Quick-Start Protocol Part 2. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.