Quick-Start Protocol March 2019

QIAseq® UPX 3' Transcriptome Library Kit

Part 1: Cell lysis and reverse transcription

Upon receipt, store the QIAseq UPX 3' Transcriptome Library Kit at –30 to –15°C. QIAseq Beads and the Cell Index (ID) RT Plate should be stored at 2–8°C.

Further information

- *QIAseq UPX 3' Transcriptome Library Handbook*: **www.qiagen.com/HB-2485**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- **Important**: Rebuffered QIAseq Beads (RQ Beads) must be used for the cleanup reactions. Consult the kit handbook for rebuffering details.
- If using the multi-use 96-well Cell ID RT Plate, consult the kit handbook.
- The recommended starting amount is 1–100 cells.
- For single cells, at least 8 cells must be multiplexed per sample index.
- If using purified RNA, consult the kit handbook.
- Ensure that the reactions are thoroughly mixed (12 times). 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 for 8 wells	Volume for 24 wells	Volume for 96 wells	Volume for 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 cell lysis premix into the required wells of the single-use Cell ID RT Plate.
3. Capture the cells into the plate containing the cell lysis premix.
4. Incubate on ice for 15 minutes.
5. Proceed immediately with the reverse transcription reaction or freeze at –80°C.

Reverse transcription

1. Prepare the RT premix on ice as described in Table 2.

Table 2. Preparation of RT premix for single-use Cell ID RT Plates

Component	Volume for 8 wells	Volume for 24 wells	Volume for 96 wells	Volume for 384 wells
3' Trans 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 1.25×10^6-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 (Thermo Fisher Scientific, cat. no. 4456739) can be added as a control. Prepare the ERCC by diluting the stock 1.25×10^6-fold using 0.1X TE Buffer.

2. Aliquot 2 µl of the RT premix into the wells.
3. Briefly centrifuge, mix by pipetting up and down, and centrifuge briefly again.

4. Incubate as follows: 10 minutes at 25°C, 90 minutes at 42°C, 15 minutes at 70°C, and hold at 4°C.
5. Upon completion of the reverse transcription reactions, combine the synthesized cDNA from the different wells into one 2 ml microcentrifuge tube.
6. Add 0.9x volumes of RQ Beads to the combined cDNA synthesis reactions as described in Table 3. Mix well by pipetting up and down 12 times.

   Important: RQ Beads (rebuffered QIAseq Beads) must be used for this cDNA cleanup step. Consult the kit handbook for rebuffering details.

Table 3. Addition of RQ Beads for cDNA cleanup

Number of wells combined	Nuclease-Free Water	RQ Bead† volume
8	60 µl	90 µl
24	0 µl	108 µl
96*	0 µl	432 µl

* When working with 384 wells, perform the cleanup in 4 sets of 96 wells. Combine supernatants after step 13.
† Rebuffered QIAseq Beads; consult the kit handbook for details.

7. Incubate for 10 minutes at room temperature (15–25°C).
8. Place the tube on a magnetic rack for 5 minutes. After the solution has cleared, leave the tube on the magnetic stand, and carefully remove and discard the supernatant.
9. Add 200 µl of 80% ethanol. Rotate the tube 3 times. Carefully remove the ethanol wash.
10. Repeat the ethanol wash in step 8. Completely remove all traces of the ethanol wash.

    Important: Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol by pipetting.

11. Air dry the opened tube on the magnetic stand at room temperature (15–25°C) for 10 minutes.

    Note: Visually inspect that the pellet is completely dry.
12. Remove the tube from the magnetic stand and elute the DNA from the beads by adding 25 µl of Nuclease-Free Water. Mix well by pipetting.
13. Return the tube to the magnetic rack until the solution has cleared.
14. Transfer 23 µl of the supernatant to clean microcentrifuge tubes.

    Important: When working with 384 wells, combine all 4 eluates to give 92 µl.

15. Adjust the supernatant volume to 100 µl using Nuclease-Free Water.
16. Add 0.9x volumes of RQ Beads. Mix well by pipetting up and down 12 times.
17. Incubate for 10 minutes at room temperature (15–25°C).
18. Place the tubes onto a magnetic rack for 5 minutes. After the solution has cleared, carefully remove and discard the supernatant.
19. Add 200 µl 80% ethanol. Rotate the tube 3 times. Carefully discard the wash.
20. Repeat the ethanol wash in step 18. Completely remove all traces of the ethanol wash.

    Important: Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol by pipetting.
21. Air dry the opened tube on the magnetic stand at room temperature (15–25°C) for 10 minutes.

    Note: Visually inspect that the pellet is completely dry.
22. Remove the tube from the magnetic stand and elute the DNA from the beads by adding 13 µl Nuclease-Free Water. Mix well by pipetting.
23. Return the tube to the magnetic rack until the solution has cleared.
24. Transfer 11 µl of the supernatant to a clean microcentrifuge tube.
25. From this point forward in the protocol, the procedures assume that all cDNA wells (either 8, 24, 96, or 384) have been combined into a single microcentrifuge tube.
26. Proceed with "Part 2: Template amplification and fragmentation, end-repair and A-addition". Alternatively, store the samples 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.