Quick-Start Protocol October 2017

QIAseq™ Immune Repertoire RNA Library Kit

Part 1: RT primer hybridization, reverse transcription, second strand synthesis, end-repair, A-addition

Further information

- *QIAseq Immune Repertoire RNA Library Kit Handbook*: **www.qiagen.com/HB-2479**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare the reagents as described in the handbook.
- **Important**: Ensure reactions are thoroughly mixed (7 to 8 times unless otherwise stated), prepared and incubated at recommended temperatures.

RT primer hybridization

1. Pre-heat a thermal cycler to 65°C with a heated lid (set at 103°C).
2. On ice, prepare the RT primer hybridization reactions as described in Table 1.

Table 1. Preparation of RT primer hybridization reactions

	1 reaction (µl)
RNA sample (10–1000 ng)	Variable
TCR RT Primer	1
Nuclease-free water	Variable
Total	**6**

3. Transfer the tube from ice to the pre-heated thermal cycler, and incubate for 5 min at 65°C followed by ice for at least 2 min.
4. Upon completion, proceed with "Reverse transcription".

Reverse transcription

5. On ice, prepare the reverse transcription reactions as described in Table 2.

Table 2. Preparation of reverse transcription reactions

	1 reaction (µl)
RT primer hybridization reaction (already in tube)	6
BC3 buffer, 5x	2
RNase Inhibitor	1
EZ Reverse Transcriptase*	1
Total	**10**

* When working with RNA amounts ≤20 ng, dilute 1 µl of the EZ Reverse Transcriptase to 5 µl using 4 µl of nuclease-free water. Pipet up and down 7 to 8 times to mix. Then add 1 µl to the reaction.

6. Incubate the tube in a thermal cycler with a heated lid (103°C) according to Table 3.

Table 3. Thermal cycler settings for reverse transcription reactions

Step	Temperature	Time
1	42°C	30 min
2	70°C	15 min
3	4°C	Hold

7. Upon completion, proceed with "Second strand synthesis". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Second strand synthesis

8. On ice, prepare the second strand reactions as described in Table 4.

Table 4. Preparation of second strand synthesis reactions

	1 reaction (µl)
Reverse-transcription reaction (already in tube)	10
XC buffer	2
RH RNase	1
dNTP	1
BX enzyme	1
Nuclease-free water	5
Total	**20**

9. Incubate the tube in a thermal cycler with a heated lid (103°C) as described in Table 5.

Table 5. Thermal cycler settings for second strand synthesis

Step	Temperature	Time
1	37°C	7 min
2	65°C	10 min
3	80°C	10 min
4	4°C	Hold

10. Upon completion, proceed with "End-repair and A-addition".

End-repair and A-addition

11. On ice, prepare the end-repair and A-addition reactions as described in Table 6.

Table 6. Preparation of end-repair and A-addition reactions

	1 reaction (µl)
Second strand product from previous section	20
ERA Buffer, 10x	5
Nuclease-free water	15
Total	**40**

12. On ice, add 10 µl ERA Enzyme to each reaction. Keep the reactions on ice until Step 15.
13. Program a thermal cycler as described in Table 7. Set the heated lid to 70°C.

    Note: If using a non-temperature-controlled lid, run with cycler lid open for step 2 and seal the strip or plate well. When the cycler reaches step 3, close the lid to avoid evaporation. Centrifuge after the run to remove any condensation.
14. Prior to adding the tubes/plate to the thermal cycler, start the program. When the thermal cycler reaches 4°C, pause the program.
15. Transfer the tubes/plate from step 12 to the pre-chilled cycler and resume the program.

Table 7. Thermal cycler settings for End-Repair and A-Addition

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	20°C	30 min
3	65°C	30 min
4	4°C	Hold

16. Upon completion, immediately proceed with “Adapter ligation” in Quick-Start Protocol Part 2.

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