Single Cell RNA

Extended Throughput Kit v1.1

Protocol

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Legal Notices

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Required Materials

ScaleBio™ Single Cell RNA Extended Throughput Kit v1.1 (PN 936360) Contents:

Kit Module	Consumable	Part Number	Qty	Cap Color	Storage Temp
RNA Extended Throughput Tagment and i5 Index PCR Module (PN 936061)	Second Strand Buffer Conc.	202110009	1	Purple	-20°C
	Second Strand Enzyme Mix	202110010	1	Purple	-20°C
	Cleanup Enzyme v1.1	935999	1	Brown	-20°C
	Tagment Buffer Conc.	202100003	1	Red	-20°C
	Tagment Enzyme Mix	202100004	1	Red	-20°C
	Index PCR Enzyme Mix	202110012	2	Orange	-20°C
	Adaptor Primer i5 Barcode Plate	936013	1	-	-20°C
	Elution Buffer	202110014	1	Clear	-20°C
RNA Extended Throughput Workflow Consumables Module (PN 935985)	Tagment Stop Solution	202110017	1	Black	RT
	Index PCR Additive	202110016	1	Orange	RT
	Final Distribution Plate	202110018	1	-	RT
RNA Extended Throughput Adaptor Primer Module (PN 935986)	Adaptor Primer i7-2 Tube	936002	1	Orange	-20°C
	Adaptor Primer i7-3 Tube	936003	1	Orange	-20°C
	Adaptor Primer i7-4 Tube	936362	1	Orange	-20°C

Required Cell Numbers

The 96-well Final Distribution Plate of the ScaleBio Single Cell RNA Sequencing Kit v1.1 is loaded with **1,600 cells per well** for a **total of 154,000 cells**. The unutilized remaining pooled cell suspension can be stored at -80°C until ready to process through the Extended Throughput Protocol.

As the number of leftover cells may vary depending on the sample type processed, Table 1 helps to determine the amount of additional Final Distribution Plates that can be filled to capture the remaining pooled cells.

Note: The number of cells required to load the additional Final Distribution Plates are the absolute minimum cells that are needed. Users should incorporate an excess of cells for better ease of handling when planning their RNA Extended Throughput v1.1 experiment.

Table 1: Cell Number Requirements

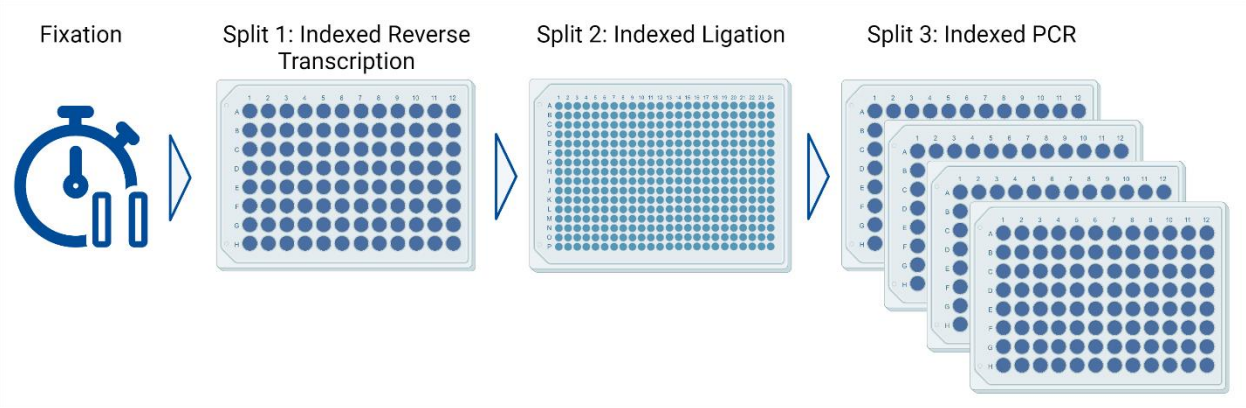
	RNA Kit v1.1	RNA Extended Throughput Kit v1.1		
		1 FD	2 FD	3 FD
RNA Extended Throughput Adaptor Primer Module	-	1	1	1
RNA Extended Throughput Tagment and i5 Index PCR Module	-	1	2	3
RNA Extended Throughput Workflow Consumables Module	-	1	2	3
Minimum Total Cells Required to Load Final Distribution Plate(s)	154,000	308,000	462,000	616,000
Estimated Cells Recovered for Sequencing	125,000	250,000	375,000	500,000

Assay Introduction

The ScaleBio™ Single Cell RNA Sequencing Kit v1.1 allows the recovery of ~125,000 cells, which can be increased with the add-on protocol from the ScaleBio Single Cell RNA Extended Throughput Kit v1.1.

After the addition of the first indexed barcode by reverse transcription, the cells are pooled and subsequently split onto the 384-well Ligation Barcode Plate where the second indexed barcode is added by ligation. The cells are then pooled again and counted to normalize the cell input to 1,600 cells per well going into the third split on the 96-well Final Distribution Plate (Figure 1).

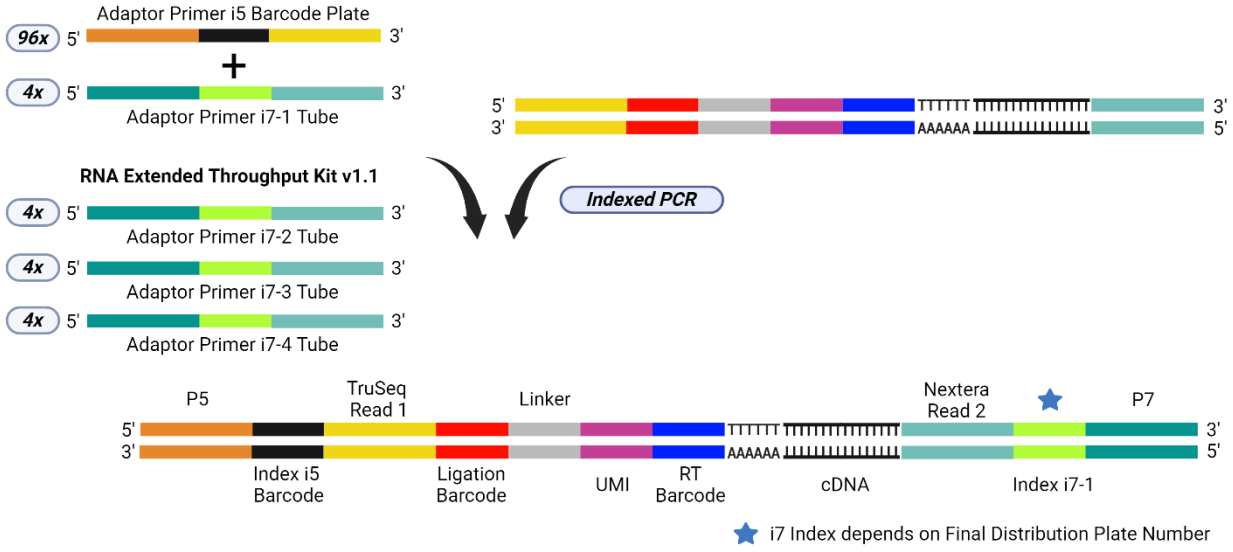
Figure 1: Procedure overview



This normalization step creates a leftover of pooled cells which may vary depending on the sample type processed.

With the ScaleBio Single Cell RNA Extended Throughput v1.1 Kit, these leftover pooled cells may be used to fill additional Final Distribution Plates on Split 3 to increase the cell recovery of the assay overall. This is achieved by providing additional unique i7 indices that are added to the libraries during the Indexed PCR step (Figure 2).

Figure 2: Primer Combinations during Indexed PCR for Extended Throughput



For correct downstream sample demultiplexing during data analysis, it is important to note which Adaptor Primer i7 is used for each Final Distribution Plate that is being processed.

The throughput of the recovered cells can be increased incrementally up to ~500,000 cells by either one, two or three Extended Throughput Kits, respectively. Please review chapter *Required Cell Numbers* for considerations on the input as well as output cell number requirements for the add-on protocol.

The ScaleBio Single Cell RNA Extended Throughput v1.1 Protocol provides instructions on processing these additional Extended Throughput Kit. This protocol should be used in conjunction with the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol as the initial steps of reverse transcription and ligation remain the same.

Please contact support@scale.bio or your local Field Application Scientist for assistance with this workflow.

Step 1: Initial Distribution and Reverse Transcription

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.
- **At Step 4**: Load **10,000 cells per well** on the initial RT Barcode Plate (**5 µL** of cell suspension with a concentration of **2000 cells per µL**) to ensure high cell numbers after ligation for loading Extended Throughput Kits.

Step 2: Ligation

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.

Step 3: Final Distribution

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	Single Cell RNA Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Workflow Consumables Module	Final Distribution Plate	1	2	3

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.
- Use the contents of the ScaleBio Single Cell RNA Extended Throughput Kit v1.1 modules listed above.
- **At Step 3**: repeat the process from step 3 onwards to load the required amount of additional Final Distribution Plates with the leftover cell suspension. The cell suspension is already diluted in Wash Buffer at a final concentration of **400 cells per µL**.

Step 4: Second Strand Synthesis and Cleanup Enzyme Digestion

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	Single Cell RNA Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Tagment and i5 Index PCR Module	Second Strand Buffer Conc.	1	2	3
	Second Strand Enzyme Mix	1	2	3
	Cleanup Enzyme v1.1	1	2	3

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.
- Use the contents of the ScaleBio Single Cell RNA Extended Throughput Kit v1.1 modules listed above.

Step 5: Tagmentation and Index PCR

Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	Single Cell RNA Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Workflow Consumables Module	Tagment Stop Solution	1	2	3
	Index PCR Additive	1	2	3
RNA Extended Throughput Tagment and i5 Index PCR Module	Tagment Buffer Conc.	1	2	3
	Tagment Enzyme Mix	1	2	3
	Index PCR Enzyme Mix	2	4	6
	Adaptor Primer i5 Barcode Plate	1	2	3
RNA Extended Throughput Adaptor Primer Module	Adaptor Primer **i7-X** Tube	AP-i7-2	AP-i7-3	AP-i7-4
		936002	936003	936362

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.
- Use the contents of the ScaleBio Single Cell RNA Extended Throughput Kit v1.1 modules listed above.
- **At Step 18**: prepare one Index PCR Master Mix for each Extended Throughput Kit that is being processed. Substitute *Adaptor Primer i7-X* in Table 2 with one of the following reagent tubes:
    - Adaptor Primer i7-2
    - Adaptor Primer i7-3
    - Adaptor Primer i7-4

Table 2: Index PCR Master Mix

Reagent	Volume (µL)
Index PCR Additive	240
Adaptor Primer i7-X	48
Index PCR Enzyme Mix	2400
Total Volume	**2688**

Caution: Write down each Adaptor Primer i7 used for each Final Distribution Plate.

- **At Step 22**: use one Adaptor Primer i5 Barcode Plate for each Extended Throughput Kit that is being processed. The plate contains 96 unique barcodes that are identical on each Adaptor Primer i5 Barcode Plate.

Step 6: Index PCR Purification

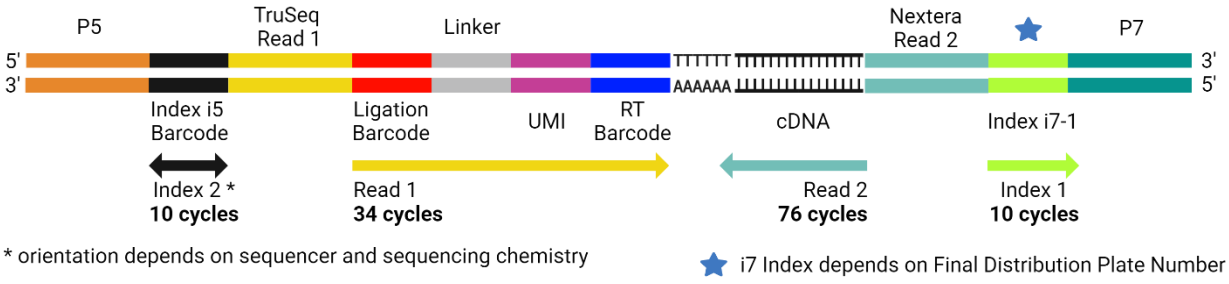
Please review the required modules and reagents from the table below depending on the required cell throughput extension:

Source	Single Cell RNA Extended Throughput Kit v1.1			
		1 FD	2 FD	3 FD
RNA Extended Throughput Tagment and i5 Index PCR Module	Elution Buffer	1	2	3

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.
- Use the contents of the ScaleBio Single Cell RNA Extended Throughput Kit v1.1 modules listed above.

Step 8: Sequencing Parameters

- Follow the instructions as described in the ScaleBio Single Cell RNA Sequencing Kit v1.1 Protocol.
- The combination of i7 Indices used for the RNA Libraries depends on the number of processed Final Distribution Plates utilized in the Extended Throughput Protocol (Table 3).



Caution: To ensure correct sample demultiplexing, libraries processed by multiple ScaleBio Single Cell RNA Extended Throughput Kits require both the i5 and the i7 indices to be sequenced.

Table 3: Index i7 Combinations for the RNA Extended Throughput Kit v1.1

Final Distribution	RNA Library	
	Reagent	PN
Plate 2	Adaptor Primer i7-2	936002
Plate 3	Adaptor Primer i7-3	936003
Plate 4	Adaptor Primer i7-4	936362

Document Revision History

Revision	Revision Date	Document ID	Changes
Rev A	Nov 2023	1020800	Initial release.
Rev B	Jul 2024	1020800	Update consumables storage temperature.