

## SMART-Seq® ICELL8® Indexing Primer Set – B

**Catalog No.**  
640218

**Amount**  
1 chip

**Lot Number**  
Specified on product label.

### Description

The SMART-Seq ICELL8 Indexing Primer Set – B enables users to analyze full-length transcripts using the SMARTer™ ICELL8 cx Single-Cell System or the SMARTer ICELL8 Single-Cell System. The SMART-Seq ICELL8 Indexing Primer Set – B contains sufficient indexes to process samples on one chip with one SMART-Seq ICELL8 Reagent Kit (1-chip size). The indexing primers from this set and SMART-Seq ICELL8 Indexing Primer Set – A (Cat. No. 640205) can be used on different ICELL8 chips for SMART-seq application. The libraries can be pooled for sequencing.

This protocol leverages SMART® technology for full-length cDNA synthesis and Nextera® technology for Illumina® library preparation with dual 72 x 72 indexes (Illumina Nextera XT library preparation kit not included). The protocol generates indexed libraries that are ready for sequencing on Illumina platforms.

### Package Contents

This primer set contains two boxes in total. Each box contains a 384-well plate prealiquoted with indexes (20 µl per well). Box 1 includes Plate 1 (ST1849) containing 72 forward indexes (1–72), and Box 2 includes Plate 2 (ST2044) containing 72 reverse indexes (73–144).

#### Plate 1 (SMART-Seq ICELL8 Forward Indexing Primer Set 1–72; ST1849)

Well position	Index name
A5	SMART-Seq ICELL8 Forward Index 1
B5	SMART-Seq ICELL8 Forward Index 2
C5	SMART-Seq ICELL8 Forward Index 3
D5	SMART-Seq ICELL8 Forward Index 4
E5	SMART-Seq ICELL8 Forward Index 5
F5	SMART-Seq ICELL8 Forward Index 6
G5	SMART-Seq ICELL8 Forward Index 7
H5	SMART-Seq ICELL8 Forward Index 8
I5	SMART-Seq ICELL8 Forward Index 9
J5	SMART-Seq ICELL8 Forward Index 10
K5	SMART-Seq ICELL8 Forward Index 11
L5	SMART-Seq ICELL8 Forward Index 12
M5	SMART-Seq ICELL8 Forward Index 13
N5	SMART-Seq ICELL8 Forward Index 14
O5	SMART-Seq ICELL8 Forward Index 15
P5	SMART-Seq ICELL8 Forward Index 16
A6	SMART-Seq ICELL8 Forward Index 17
B6	SMART-Seq ICELL8 Forward Index 18

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# Certificate of Analysis

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## SMART-Seq® ICELL8® Indexing Primer Set – B

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C6	SMART-Seq ICELL8 Forward Index 19
D6	SMART-Seq ICELL8 Forward Index 20
E6	SMART-Seq ICELL8 Forward Index 21
F6	SMART-Seq ICELL8 Forward Index 22
G6	SMART-Seq ICELL8 Forward Index 23
H6	SMART-Seq ICELL8 Forward Index 24
I6	SMART-Seq ICELL8 Forward Index 25
J6	SMART-Seq ICELL8 Forward Index 26
K6	SMART-Seq ICELL8 Forward Index 27
L6	SMART-Seq ICELL8 Forward Index 28
M6	SMART-Seq ICELL8 Forward Index 29
N6	SMART-Seq ICELL8 Forward Index 30
O6	SMART-Seq ICELL8 Forward Index 31
P6	SMART-Seq ICELL8 Forward Index 32
A7	SMART-Seq ICELL8 Forward Index 33
B7	SMART-Seq ICELL8 Forward Index 34
C7	SMART-Seq ICELL8 Forward Index 35
D7	SMART-Seq ICELL8 Forward Index 36
E7	SMART-Seq ICELL8 Forward Index 37
F7	SMART-Seq ICELL8 Forward Index 38
G7	SMART-Seq ICELL8 Forward Index 39
H7	SMART-Seq ICELL8 Forward Index 40
I7	SMART-Seq ICELL8 Forward Index 41
J7	SMART-Seq ICELL8 Forward Index 42
K7	SMART-Seq ICELL8 Forward Index 43
L7	SMART-Seq ICELL8 Forward Index 44
M7	SMART-Seq ICELL8 Forward Index 45
N7	SMART-Seq ICELL8 Forward Index 46
O7	SMART-Seq ICELL8 Forward Index 47
P7	SMART-Seq ICELL8 Forward Index 48
A8	SMART-Seq ICELL8 Forward Index 49
B8	SMART-Seq ICELL8 Forward Index 50
C8	SMART-Seq ICELL8 Forward Index 51
D8	SMART-Seq ICELL8 Forward Index 52
E8	SMART-Seq ICELL8 Forward Index 53
F8	SMART-Seq ICELL8 Forward Index 54
G8	SMART-Seq ICELL8 Forward Index 55
H8	SMART-Seq ICELL8 Forward Index 56
I8	SMART-Seq ICELL8 Forward Index 57
J8	SMART-Seq ICELL8 Forward Index 58
K8	SMART-Seq ICELL8 Forward Index 59
L8	SMART-Seq ICELL8 Forward Index 60
M8	SMART-Seq ICELL8 Forward Index 61
N8	SMART-Seq ICELL8 Forward Index 62
O8	SMART-Seq ICELL8 Forward Index 63

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## SMART-Seq® ICELL8® Indexing Primer Set – B

P8	SMART-Seq ICELL8 Forward Index 64
A9	SMART-Seq ICELL8 Forward Index 65
B9	SMART-Seq ICELL8 Forward Index 66
C9	SMART-Seq ICELL8 Forward Index 67
D9	SMART-Seq ICELL8 Forward Index 68
A10	SMART-Seq ICELL8 Forward Index 69
B10	SMART-Seq ICELL8 Forward Index 70
C10	SMART-Seq ICELL8 Forward Index 71
D10	SMART-Seq ICELL8 Forward Index 72

## Plate 2 (SMART-Seq ICELL8 Reverse Indexing Primer Set 73–144; ST2044)

Well position	Index name
A13	SMART-Seq ICELL8 Reverse Index 73
B13	SMART-Seq ICELL8 Reverse Index 74
C13	SMART-Seq ICELL8 Reverse Index 75
D13	SMART-Seq ICELL8 Reverse Index 76
E13	SMART-Seq ICELL8 Reverse Index 77
F13	SMART-Seq ICELL8 Reverse Index 78
G13	SMART-Seq ICELL8 Reverse Index 79
H13	SMART-Seq ICELL8 Reverse Index 80
I13	SMART-Seq ICELL8 Reverse Index 81
J13	SMART-Seq ICELL8 Reverse Index 82
K13	SMART-Seq ICELL8 Reverse Index 83
L13	SMART-Seq ICELL8 Reverse Index 84
M13	SMART-Seq ICELL8 Reverse Index 85
N13	SMART-Seq ICELL8 Reverse Index 86
O13	SMART-Seq ICELL8 Reverse Index 87
P13	SMART-Seq ICELL8 Reverse Index 88
A14	SMART-Seq ICELL8 Reverse Index 89
B14	SMART-Seq ICELL8 Reverse Index 90
C14	SMART-Seq ICELL8 Reverse Index 91
D14	SMART-Seq ICELL8 Reverse Index 92
E14	SMART-Seq ICELL8 Reverse Index 93
F14	SMART-Seq ICELL8 Reverse Index 94
G14	SMART-Seq ICELL8 Reverse Index 95
H14	SMART-Seq ICELL8 Reverse Index 96
I14	SMART-Seq ICELL8 Reverse Index 97
J14	SMART-Seq ICELL8 Reverse Index 98
K14	SMART-Seq ICELL8 Reverse Index 99
L14	SMART-Seq ICELL8 Reverse Index 100
M14	SMART-Seq ICELL8 Reverse Index 101
N14	SMART-Seq ICELL8 Reverse Index 102
O14	SMART-Seq ICELL8 Reverse Index 103
P14	SMART-Seq ICELL8 Reverse Index 104
A15	SMART-Seq ICELL8 Reverse Index 105

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## SMART-Seq® ICELL8® Indexing Primer Set – B

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B15	SMART-Seq ICELL8 Reverse Index 106
C15	SMART-Seq ICELL8 Reverse Index 107
D15	SMART-Seq ICELL8 Reverse Index 108
E15	SMART-Seq ICELL8 Reverse Index 109
F15	SMART-Seq ICELL8 Reverse Index 110
G15	SMART-Seq ICELL8 Reverse Index 111
H15	SMART-Seq ICELL8 Reverse Index 112
I15	SMART-Seq ICELL8 Reverse Index 113
J15	SMART-Seq ICELL8 Reverse Index 114
K15	SMART-Seq ICELL8 Reverse Index 115
L15	SMART-Seq ICELL8 Reverse Index 116
M15	SMART-Seq ICELL8 Reverse Index 117
N15	SMART-Seq ICELL8 Reverse Index 118
O15	SMART-Seq ICELL8 Reverse Index 119
P15	SMART-Seq ICELL8 Reverse Index 120
A16	SMART-Seq ICELL8 Reverse Index 121
B16	SMART-Seq ICELL8 Reverse Index 122
C16	SMART-Seq ICELL8 Reverse Index 123
D16	SMART-Seq ICELL8 Reverse Index 124
E16	SMART-Seq ICELL8 Reverse Index 125
F16	SMART-Seq ICELL8 Reverse Index 126
G16	SMART-Seq ICELL8 Reverse Index 127
H16	SMART-Seq ICELL8 Reverse Index 128
I16	SMART-Seq ICELL8 Reverse Index 129
J16	SMART-Seq ICELL8 Reverse Index 130
K16	SMART-Seq ICELL8 Reverse Index 131
L16	SMART-Seq ICELL8 Reverse Index 132
M16	SMART-Seq ICELL8 Reverse Index 133
N16	SMART-Seq ICELL8 Reverse Index 134
O16	SMART-Seq ICELL8 Reverse Index 135
P16	SMART-Seq ICELL8 Reverse Index 136
A17	SMART-Seq ICELL8 Reverse Index 137
B17	SMART-Seq ICELL8 Reverse Index 138
C17	SMART-Seq ICELL8 Reverse Index 139
D17	SMART-Seq ICELL8 Reverse Index 140
A18	SMART-Seq ICELL8 Reverse Index 141
B18	SMART-Seq ICELL8 Reverse Index 142
C18	SMART-Seq ICELL8 Reverse Index 143
D18	SMART-Seq ICELL8 Reverse Index 144

### Storage Conditions

- Store at  $-70^{\circ}\text{C}$ .

### Shelf Life

- 1 year from date of receipt under proper storage conditions.

## Shipping Conditions

- Dry ice (–70°C)

## Product Documents

Documents for our products are available for download at [takarabio.com/manuals](http://takarabio.com/manuals)

The following documents apply to this product:

- SMARTer ICELL8 SMART-Seq User Manual

## Quality Control Data

All index primers were verified by ESI analysis to have a major peak with a measured molecular weight within 10 Da of the predicted molecular weight.

A sample kit from each lot was tested on the SMARTer ICELL8 Single-Cell System to ensure that all indexes (72 forward indexes and 72 reverse indexes) were in the correct wells in plates by using each index three times as follows:

10 pg of Control K-562 RNA was dispensed into 216 nanowells and subjected to double-stranded cDNA synthesis, PCR with eight cycles, tagmentation, and library PCR1 with six cycles, as described in the SMARTer ICELL8 SMART-Seq User Manual. The PCR product was collected from the chip, purified with the Agencourt AMPure XP PCR purification system (Beckman Coulter, Cat. No. A63880), and then subjected to a final library PCR2 with seven cycles. The PCR product (library) was purified again with AMPure beads and eluted in 17 µl of Elution Buffer.

The yield of the resulting library was >150 ng, determined using a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat. No. Q32851) on a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Cat. No. Q32866). The average library size was 400–800 bp, determined using an Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626) on an Agilent 2100 Bioanalyzer system (Agilent, Cat. No. G2939BA). The library was denatured, diluted to 10 pM and sequenced on a MiSeq® sequencer using the MiSeq Reagent Kit v3 (150-cycle) (Illumina, Cat. No. MS-102-3001) as follows: Read 1 = 75 cycles, Read 2 = 75 cycles, Forward index (i5) = 8 cycles, and Reverse index (i7) = 8 cycles.

The sequencing reads were de-multiplexed and counted using Takara Bio's scRNA-Seq analysis pipeline, mappa™ software. The average of number of reads per index was calculated and confirmed to be within 1,000-fold difference in >98% of indexes. In addition, the average of reads from the negative controls (without RNA) was confirmed to be <0.25% reads.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

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### NOTICE TO PURCHASER:

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