

# Certificate of Analysis

## SMART-Seq® Pro Indexing Primer Set - A

**Catalog No.**  
640258

**Amount**  
1 chip

**Lot Number**  
Specified on product label.

### Description

The SMART-Seq Pro Indexing Primer Set - A consists of one 384-well plate containing 72 forward and 72 reverse index primers, prealiquoted and dried down for more convenient storage. Primer quantities are sufficient to process samples on one ICELL8® cx nanowell chip using the SMART-Seq Pro protocol or can be resuspended to custom concentrations for user-generated protocols. These index primers, together with SMART-Seq Pro Indexing Primer Set - B (Cat. No. 640260), allow two SMART-Seq Pro libraries to be multiplexed in a single sequencing run.

### Package Contents

- 1 Plate Sealing Film
- 1 384-well plate with index primers. Each nanowell contains 0.05 nmol of a specific primer. The association of index primer with well location on the plate is listed in the table below (continues for the next three pages).

Well position	Index
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
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

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<b>Well position</b>	<b>Index</b>
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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<b>Well position</b>	<b>Index</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
A13	SMART-Seq ICELL8 Reverse Index 1
B13	SMART-Seq ICELL8 Reverse Index 2
C13	SMART-Seq ICELL8 Reverse Index 3
D13	SMART-Seq ICELL8 Reverse Index 4
E13	SMART-Seq ICELL8 Reverse Index 5
F13	SMART-Seq ICELL8 Reverse Index 6
G13	SMART-Seq ICELL8 Reverse Index 7
H13	SMART-Seq ICELL8 Reverse Index 8
I13	SMART-Seq ICELL8 Reverse Index 9
J13	SMART-Seq ICELL8 Reverse Index 10
K13	SMART-Seq ICELL8 Reverse Index 11
L13	SMART-Seq ICELL8 Reverse Index 12
M13	SMART-Seq ICELL8 Reverse Index 13
N13	SMART-Seq ICELL8 Reverse Index 14
O13	SMART-Seq ICELL8 Reverse Index 15
P13	SMART-Seq ICELL8 Reverse Index 16
A14	SMART-Seq ICELL8 Reverse Index 17
B14	SMART-Seq ICELL8 Reverse Index 18
C14	SMART-Seq ICELL8 Reverse Index 19
D14	SMART-Seq ICELL8 Reverse Index 20
E14	SMART-Seq ICELL8 Reverse Index 21
F14	SMART-Seq ICELL8 Reverse Index 22
G14	SMART-Seq ICELL8 Reverse Index 23
H14	SMART-Seq ICELL8 Reverse Index 24
I14	SMART-Seq ICELL8 Reverse Index 25
J14	SMART-Seq ICELL8 Reverse Index 26
K14	SMART-Seq ICELL8 Reverse Index 27
L14	SMART-Seq ICELL8 Reverse Index 28
M14	SMART-Seq ICELL8 Reverse Index 29
N14	SMART-Seq ICELL8 Reverse Index 30
O14	SMART-Seq ICELL8 Reverse Index 31
P14	SMART-Seq ICELL8 Reverse Index 32

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<b>Well position</b>	<b>Index</b>
A15	SMART-Seq ICELL8 Reverse Index 33
B15	SMART-Seq ICELL8 Reverse Index 34
C15	SMART-Seq ICELL8 Reverse Index 35
D15	SMART-Seq ICELL8 Reverse Index 36
E15	SMART-Seq ICELL8 Reverse Index 37
F15	SMART-Seq ICELL8 Reverse Index 38
G15	SMART-Seq ICELL8 Reverse Index 39
H15	SMART-Seq ICELL8 Reverse Index 40
I15	SMART-Seq ICELL8 Reverse Index 41
J15	SMART-Seq ICELL8 Reverse Index 42
K15	SMART-Seq ICELL8 Reverse Index 43
L15	SMART-Seq ICELL8 Reverse Index 44
M15	SMART-Seq ICELL8 Reverse Index 45
N15	SMART-Seq ICELL8 Reverse Index 46
O15	SMART-Seq ICELL8 Reverse Index 47
P15	SMART-Seq ICELL8 Reverse Index 48
A16	SMART-Seq ICELL8 Reverse Index 49
B16	SMART-Seq ICELL8 Reverse Index 50
C16	SMART-Seq ICELL8 Reverse Index 51
D16	SMART-Seq ICELL8 Reverse Index 52
E16	SMART-Seq ICELL8 Reverse Index 53
F16	SMART-Seq ICELL8 Reverse Index 54
G16	SMART-Seq ICELL8 Reverse Index 55
H16	SMART-Seq ICELL8 Reverse Index 56
I16	SMART-Seq ICELL8 Reverse Index 57
J16	SMART-Seq ICELL8 Reverse Index 58
K16	SMART-Seq ICELL8 Reverse Index 59
L16	SMART-Seq ICELL8 Reverse Index 60
M16	SMART-Seq ICELL8 Reverse Index 61
N16	SMART-Seq ICELL8 Reverse Index 62
O16	SMART-Seq ICELL8 Reverse Index 63
P16	SMART-Seq ICELL8 Reverse Index 64
A17	SMART-Seq ICELL8 Reverse Index 65
B17	SMART-Seq ICELL8 Reverse Index 66
C17	SMART-Seq ICELL8 Reverse Index 67
D17	SMART-Seq ICELL8 Reverse Index 68
A18	SMART-Seq ICELL8 Reverse Index 69
B18	SMART-Seq ICELL8 Reverse Index 70
C18	SMART-Seq ICELL8 Reverse Index 71
D18	SMART-Seq ICELL8 Reverse Index 72

## Storage Conditions

- Store at Room temperature.

## Expiration Date

- Specified on product label.

## Shipping Conditions

- Room temperature

## 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:

- SMART-Seq Pro Application 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 ICELL8 cx Single-Cell System to ensure that all 144 indexes (72 forward and 72 reverse) were in the correct wells in plates by using each index three times in the following procedure:

2 pg of Control K-562 RNA was dispensed into 216 nanowells and subjected to double-stranded cDNA synthesis, PCR1 with fourteen cycles, tagmentation, and PCR2 with five cycles, as described in the SMART-Seq Pro Application Kit User Manual. The PCR product was collected from the chip, purified with NucleoMag NGS Clean-up and Size Select (Cat. No. 744970.50), and then subjected to a final PCR (PCR3) with five cycles. The PCR product (library) was purified again with NucleoMag NGS Clean-up and Size Select and eluted in 17 µl of Elution Buffer.

The yield of the resulting library was 22–108 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 library was quantified by qPCR with a Library Quantification Kit (Cat. No. 638324), denatured, 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 demultiplexed and counted using Cogent™ NGS Analysis Pipeline. The average of number of reads per index was calculated and confirmed to be within a 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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### CATALOG NO.

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### NOTICE TO PURCHASER:

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1/10/2022