Certificate of Analysis

SMART-Seq® Pro Indexing Primer Set - B

Catalog No.	Amount	Lot Number
640260	1 chip	Specified on product label.

Description

The SMART-Seq Pro Indexing Primer Set - B 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 - A (Cat. No. 640258), 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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Well position	Index
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

Well position	Index
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 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

Well position	Index
A15	SMART-Seq ICELL8 Reverse Index 105
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 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**
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.

Notice to Purchaser

SMART-Seq® Pro Indexing Primer Set - B

CATALOG NO.

640260

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, and described on the product´s web page at http://www.takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

TRADEMARKS:

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