Certificate of Analysis

SMART-Seq® ICELL8® Reagent Kit

Catalog Nos.	Amount	Lot Number
640202	1 chip	Specified on product label.
640203	3 chips	Specified on product label.
640204	5 chips	Specified on product label.

Description

For the ICELL8 Single-Cell System (Cat. No. 640000):

SMART-Seq ICELL8 reagent kits, available for purchase separately, combine with ICELL8 250v chips (Cat. No. 640183) and the SMART-Seq ICELL8 Indexing Primer Set – A (Cat. No. 640205) to create the SMART-Seq ICELL8 application kit protocol, which enables users to analyze full-length transcripts using the ICELL8 Single-Cell System.

For the ICELL8 cx Single-Cell System (Cat. No. 640188):

SMART-Seq ICELL8 reagent kits, available for purchase separately, combine with ICELL8 350v chips (Cat. No. 640019) and the SMART-Seq ICELL8 Indexing Primer Set – A (Cat. No. 640205) to create the SMART-Seq ICELL8 cx application kit protocol, which enables users to analyze full-length transcripts using the ICELL8 cx Single-Cell System.

Both protocols leverage SMART® technology for full-length cDNA synthesis and Nextera® technology for Illumina® library preparation with dual 72 x 72 indexes (Nextera Tagment DNA Enzyme not included). The protocols generate indexed libraries that are ready for sequencing on Illumina platforms.

Package Contents

Package 1:

640202 (1 chip)	640203 (3 chips)	640204 (5 chips)	
10 µl	10 µl	10 µl	Control K-562 RNA (1 µg/µl)

Package 2:

640202 (1 chip)	640203 (3 chips)	640204 (5 chips)	
25 µl	75 µl	125 µl	ICELL8 Fiducial Mix (1X)
12 µl	36 µl	60 µl	Second Diluent (100X)
17 µl	51 µl	85 µl	RNase Inhibitor (40 U/µl)
24 µl	72 µl	120 µl	SMART-Seq ICELL8 CDS (100 µM)
9 µl	27 µl	45 µl	SMART-Seq ICELL8 Oligonucleotide (100 µM)
120 µl	360 µl	600 µl	SMART-Seq ICELL8 RT-PCR Buffer
9 µl	27 µl	45 µl	SMARTScribe™ Reverse Transcriptase (200 U/µl)
21 µl	63 µl	105 µl	Terra™ PCR Direct Polymerase Mix (1.25 U/µl)
10 µl	10 µl	10 µl	TRH (200 U/µl)

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640202 (1 chip)	640203 (3 chips)	640204 (5 chips)	
140 µl	420 µl	700 µl	$MgCl_2$
75 µl	225 µl	375 µl	SeqAmp™ CB PCR Buffer (2X)
30 µl	90 µl	150 µl	5X Primer Mix
200 µl	600 µl	1,000 µl	Elution Buffer (10 mM)
12 µl	36 µl	60 µl	BSA (1%)
200 µl	600 µl	1,000 µl	Nuclease-Free Water

Storage Conditions

- Store Control K-562 RNA (Package 1) at –70°C.
- Store all other reagents (Package 2) at –20°C.

Expiration Date

- Specified on product label.

Shipping Conditions

- Dry ice

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**

The following documents apply to the ICELL8 Single-Cell System (Cat. No. 640000):

- SMART-Seq ICELL8 Application Kit User Manual
- ICELL8 MultiSample NanoDispenser User Manual
- ICELL8 Imaging System User Manual
- CellSelect® Software User Manual

The following documents apply to the ICELL8 cx Single-Cell System (Cat. No. 640188):

- SMART-Seq ICELL8 cx Application Kit User Manual
- ICELL8 cx Single-Cell System User Manual
- ICELL8 Imaging System User Manual
- ICELL8 cx CellSelect Software User Manual

Quality Control Data

A sample kit from each lot was tested on the ICELL8 Single-Cell System, as follows, to ensure that it produces an appropriate library in terms of yield, size, number of sequencing reads, and number of genes detected:

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 SMART-Seq ICELL8 Application Kit 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 Technology, 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.

Data analysis, demultiplexing, alignment, and counting were performed using Cogent™ NGS Analysis Software, Takara Bio's scRNA-seq analysis pipeline. Results were as follows: total reads were >2 x 10^7; percentage of uniquely mapped reads (hg38) was >70%; and the average number of genes detected per unique combination of indexes was 5,000–7,500. The average percentage of uniquely mapped reads from the negative controls (without RNA) and empty wells (no dispenses except for RNA) was <0.25% and <0.1%, respectively.

Quality Control Testing	
Test Parameter	**Result**
Library yield (Qubit)	>150 ng
Library size (Bioanalyzer)	400–800 bp
Total reads	>2 x 10^7
% uniquely mapped reads (hg38)	>70%
Average number of genes detected	5,000–7,500
% uniquely mapped reads (hg38, negative control)	<0.25%
% uniquely mapped reads (hg38, empty wells)	<0.1%

ICELL8 Fiducial Mix

ICELL8 Fiducial Mix was dispensed into an ICELL8 150v Chip (Cat. No. 640013) in a predetermined pattern. The chip was imaged on the ICELL8 microscope with the Micro-Manager software and analyzed using CellSelect Software. The software must automatically detect all wells with ICELL8 Fiducial Mix present.

Second Diluent

Human K-562 cells were prepared using Second Diluent, dispensed, imaged, and analyzed according to the ICELL8 Chip and Reagent 3′ DE Kit User Manual. Cells were dispensed into an ICELL8 150v Chip (Cat. No. 640013), the chip was imaged on the ICELL8 Imaging System with Micro-Manager software, and then the images were analyzed using CellSelect Software. The number of single-cell-containing wells automatically identified by the CellSelect Software must be greater than or equal to 1,500. Percent cell viability is calculated as the number of candidate wells divided by the number of single-cell-containing wells. This number must be greater than 85% of the Moxie Population Index (MPI) measured for the cells prior to dilution for dispensing.

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

Notice to Purchaser

SMART-Seq® ICELL8® Reagent Kit

CATALOG NOS.

640202, 640203 & 640204

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.

STATEMENT 442

This Product is protected by one or more patents from the family comprising: US11479806, People's Republic of China Patent: ZL201780061724.9, DE602017050404.1, EP3538662, FR3538662, UK3538662, JP7050057, SE3538662 and any corresponding patents, divisionals, continuations, patent applications and foreign filings sharing priority with the same family.

TRADEMARKS:

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4/1/2024