

Certificate of Analysis

SMART-Seq® Pro Reagent Kit

Catalog No.	Amount	Lot Number
640259 (Not sold separately) Sold as part of 640257	2 chips	Specified on product label.

Description

SMART-Seq Pro Reagent Kit, as part of SMART-Seq Pro Application Kit - 2 Chip (Cat. No. 640257), contains the reagents enabling users to analyze full-length transcripts using the ICELL8® cx Single-Cell System (Cat. No. 640188 or 640189).

Package Contents

Package 1:

- 10 µl Control K-562 RNA (1 µg/µl)

Package 2:

- 25 µl Second Diluent (100X)
- 25 µl BSA (1%)
- 50 µl RNase Inhibitor (40 U/µl)
- 5 µl SMART-Seq Pro CDS (100 µM)
- 20 µl SMART-Seq Pro Oligonucleotide (100 µM)
- 200 µl SMART-Seq Pro RT Buffer
- 40 µl SMART-Seq Pro Lysis Buffer
- 80 µl SMARTScribe™ Reverse Transcriptase (200 U/µl)
- 60 µl SMART-Seq Pro PCR 1 Buffer
- 90 µl Terra™ Direct PCR Polymerase Mix (1.25 U/µl)
- 10 µl MgCl₂
- 100 µl SeqAmp™ CB PCR Buffer (2X)
- 40 µl 5X Primer Mix
- 350 µl Elution Buffer (10 mM)
- 500 µl Nuclease-Free Water
- 20 ml SMART-Seq Pro Index Resuspension Buffer

Package 3:

- 220 µl SMART-Seq Pro Tagmentation Buffer

Storage Conditions

- Store Control K-562 RNA at -70°C.
- Store Package 2 reagents at -20°C.
- Store SMART-Seq Pro Tagmentation Buffer at room temperature.

Takara Bio USA, Inc.

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Certificate of Analysis

SMART-Seq® Pro Reagent Kit (Not sold separately)

Cat. No. 640259

Sold as a part of 640257

Expiration Date

- Specified on product label.

Shipping Conditions

- Package 1 and Package 2: Dry ice
- Package 3: 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

A sample kit from each lot was tested on the ICCELL8 cx Single-Cell System using the following procedure to ensure that it produces an appropriate library in terms of yield, size, number of sequencing reads, and number of genes detected.

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 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 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.

Data analysis, demultiplexing, alignment, and counting were performed using Cogent™ NGS Analysis Pipeline. Results were as follows: total reads were $>1.6 \times 10^7$, the percentage of uniquely mapped reads (hg38) was $>70\%$, and the average number of genes detected per unique combination of indexes was 3,200–5,094. The average percentages of uniquely mapped reads from the negative controls (without RNA) and empty wells (no dispenses except for RNA) were $<0.25\%$ and $<0.1\%$ of total reads, respectively.

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

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CATALOG NO.

640259

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.

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STATEMENT 275

SMART-Seq2 Technology. This product is sold under exclusive license from Ludwig Institute of Cancer Research, Ltd. and is covered by US Patent No. 10266894, Japanese Patent No. 6336080, and European Patent No. 3036336, and pending U.S. patent application and/or pending claims of foreign counterparts. For license information, please contact a Takara Bio USA, Inc. licensing representative by e-mail at licensing@takarabio.com.

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