

## ICELL8® 3' DE Reagent Kit

Catalog No.	Amount	Lot Number
640167 (Also sold as a part of 640164, 640165, 640166, 640188, 640189)	1 rxn	Specified on product label.

### Description

The ICELL8 3' DE Reagent Kit is used with the ICELL8 3' DE Chip, the ICELL8 Collection Kit, and the ICELL8 Loading Kit as part of the ICELL8 Chip and Reagent 3' DE Kit, which enables Illumina® sequencing and differential gene expression analysis—via transcript 3' end counting—of single cells isolated on the ICELL8 cx Single-Cell System or ICELL8 Single-Cell System. Following staining, dilution, and dispensation of cells into the 5,184-nanowell ICELL8 3' DE Chip, CellSelect® Software is used to automatically identify wells containing single, viable cells, and to direct nanowell-specific delivery of coupled RT-PCR reagents provided in the kit—including a 5' SMART® primer provided in the ICELL8 3' DE Oligo Mix. cDNA synthesis is oligo(dT)-primed using uniquely barcoded primers that have been preprinted into each nanowell of the ICELL8 3' DE Chip, and PCR amplification of the barcoded cDNA is then performed in-chip. Full-length amplicons are pooled and purified off-chip, and sequencing libraries are prepared using a transposase-based method that enriches for cDNA derived from mRNA 3' ends followed by a limited-cycle PCR amplification step that results in incorporation of Illumina index sequences. Most importantly, the kit allows for efficient, cost-effective, and high-throughput single-cell gene expression analysis.

### Package Contents

#### Box 1:

- 5 µl Control K-562 RNA (1 µg/µl)

#### Box 2:

- 5 µl MgCl<sub>2</sub> (1 M)
- 65 µl 5X First-Strand Buffer
- 35 µl SeqAmp™ PCR Buffer (2X)
- 30 µl SMARTScribe™ Reverse Transcriptase (100 U/µl)
- 10 µl SeqAmp DNA Polymerase
- 20 µl Triton X-100 (10%)
- 5 µl ICELL8 3' DE Oligo Mix
- 10 µl DTT (100 mM)
- 60 µl GC Melt (5 M)
- 25 µl dNTP Mix (25 mM each)
- 15 µl Second Diluent (100X)
- 25 µl ICELL8 Fiducial Mix (1X)
- 15 µl RNase Inhibitor (40 U/µl)
- 10 µl Primer P5 (5 µM)
- 500 µl Nuclease-Free Water

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# Certificate of Analysis

Cat. No. 640167

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## Storage Conditions

- Store Control K-562 RNA at  $-70^{\circ}\text{C}$
- Store all other reagents at  $-20^{\circ}\text{C}$

**NOTE:** At times, precipitate may be observed in the GC Melt reagent. This precipitate does not affect the performance of the kit. The precipitate can be dissolved rapidly by mixing at room temperature or warming at  $37^{\circ}\text{C}$  for a few minutes.

## Shelf Life

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

## Shipping Conditions

- Dry ice ( $-70^{\circ}\text{C}$ )

## 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:

- ICELL8 Chip and Reagent 3' DE Kit User Manual
- ICELL8 Imaging System User Manual
- CellSelect Software User Manual

## Quality Control Data

### ICELL8 3' DE Reagent Kit

A sample kit from each lot was tested as follows: 4 ng of Control K-562 RNA was subjected to coupled full-length double-stranded cDNA synthesis and PCR for 24 cycles using the reagent mix composition described in the ICELL8 Chip and Reagent 3' DE Kit User Manual. PCR products were purified with Agencourt AMPure XP beads, and resuspended in 12  $\mu\text{l}$  of water. 1  $\mu\text{l}$  of purified PCR product was quantified using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Cat. No. Q32866) and Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat. No. Q32851). Following quantification, the PCR product was normalized to 1 ng/ $\mu\text{l}$  and analyzed using an Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626) on an Agilent Bioanalyzer. Qualitative assessment indicated a cDNA size range of approximately 400 bp to 3,000 bp, with a peak  $>1,000$  bp after manual integration. Total cDNA yield was calculated by multiplying the Bioanalyzer-measured concentration times the dilution factor. The concentration of the cDNA was confirmed to be  $>1$  ng/ $\mu\text{l}$ .

### ICELL8 Fiducial Mix

ICELL8 Fiducial Mix was dispensed into an unprinted ICELL8 150v Chip (Takara Bio, Cat. No. 640013) in a predetermined pattern. The chip was imaged on the ICELL8 Imaging System with the Micro-Manager software and analyzed using CellSelect software. It was confirmed that the software could automatically detect all wells containing ICELL8 Fiducial Mix.

### Second Diluent

K-562 cells were prepared according to the ICELL8 Chip and Reagent 3' DE Kit User Manual, including the Second Diluent. Cells were dispensed into an unprinted ICELL8 150v Chip (Takara Bio, Cat. No. 640013) according to the ICELL8 Chip and Reagent 3' DE Kit User Manual. The chip was imaged on the ICELL8 Imaging System with the Micro-Manager software and analyzed using CellSelect Software. The number of single cell-containing wells automatically identified by the CellSelect Software was confirmed to 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 was confirmed to be greater than 85% of the Moxi 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.

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### CATALOG NO.

640167

### 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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