

ICELL8® Human TCR a/b Profiling Reagent Kit

Catalog No.	Amount	Lot Number
640182	Each	Specified on product label.

Description

The ICELL8 Human TCR a/b Profiling Reagent Kit is required for the ICELL8 Human TCR a/b Profiling workflow, which enables users to analyze T-cell receptor (TCR) diversity from single T cells using the ICELL8 Single-Cell System.

The kit leverages SMART® technology and employs a 5' RACE-like approach to capture complete V(D)J variable regions of TCR transcripts. The reagents are dispensed into a ICELL8 TCR Chip (Cat. No. 640178) which includes 5,184 nanowells, containing 1,728 preprinted barcodes (each barcode is printed three times on the chip) along with primers from the ICELL8 Human TCR a/b Profiling - Indexing Set (Cat. Nos. 640179, 640180 & 640181) that incorporate Illumina®-specific adaptor sequences during cDNA amplification. The protocol generates indexed libraries that are ready for sequencing on Illumina platforms. The protocol also allows whole transcriptome library generation of 5' ends of all transcripts.

Package Contents

Package 1:

640182

(1 each)

5 µl Control Jurkat Total RNA (1 µg/µl)

Package 2:

640182

(1 each)

15 µl Second Diluent (100X)
15 µl RNase Inhibitor (40 U/µl)
25 µl ICELL8 Fiducial Mix (1X)
25 µl cDNA Amplification dNTP Mixture (25 mM each)
10 µl Triton X-100 (3%)
35 µl SMARTScribe™ Buffer (10X)
60 µl GC Melt (5 M)
10 µl MgCl₂ (1 M)
10 µl Oligo dT Amp Primer (100 µM)
10 µl DTT (100 mM)
50 µl SMARTScribe Reverse Transcriptase (100 U/µl)
35 µl cDNA Amplification Buffer (2X)

Package 2 contents continued on next page.

Takara Bio USA, Inc.

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Certificate of Analysis

Cat. No. 640182

ICELL8 Human TCR a/b Profiling Reagent Kit

10 µl	ICELL8 Amp Primer (10 µM)
10 µl	TCRa Human Primer 1 (2 µM)
10 µl	TCRb Human Primer 1 (2 µM)
12 µl	cDNA Amplification Polymerase (50X)
15 µl	P5 Primer (5 µM)
10 µl	TCR Amplification Polymerase (1.25 U/µl)
25 µl	TCR Amplification Buffer (5X)
10 µl	TCR Amplification dNTP Mixture (2.5 mM each)
500 µl	Nuclease-Free Water

Storage Conditions

- Store Control Jurkat Total RNA –70°C.
- Store all other reagents at –20°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°C for a few minutes.

Shelf Life

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

Shipping Conditions

- Dry ice (–70°C)

Product Documents

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

The following documents apply to this product:

- ICELL8 Human TCR a/b Profiling User Manual
- ICELL8 Imaging System User Manual
- ICELL8 MultiSample Nanodispenser User Manual
- CellSelect® Software User Manual

Quality Control Data

ICELL8 Human TCR a/b Profiling Reagent Kit

A sample kit from each lot was tested as follows: 2 ng of Control Jurkat RNA were subjected to coupled full-length ds cDNA synthesis and PCR for 24 cycles as described in the ICELL8 Human TCR a/b Profiling Kit User Manual. PCR products were then purified with AMPure XP beads and resuspended in 15 µl of water. 1 µl of the PCR product was quantified using a Qubit2.0 Fluorometer (Life Technologies, Cat No. Q32866) and the Qubit dsDNA HS Assay Kit (Life Technologies, Cat No. Q32851). Following quantification, the PCR product was normalized to 3–4 ng/µl and analyzed using an Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626) on an Agilent Bioanalyzer. Qualitative assessment of the cDNA determined that it was within the size range of 400 bp to 5,000 bp, and a peak >600 bp after manual integration was confirmed. Total cDNA yield was calculated by multiplying the Bioanalyzer measured concentration with a dilution factor and the elution volume. The concentration of the cDNA was confirmed to be >4 ng/µl. 500 pg of cDNA was used as the template in the first TCR-specific PCR for 16 cycles. 1 µl of the product from this PCR was then used as a template in a second TCR-specific PCR for 14 cycles. The PCR products from the second PCR were purified with AMPure XP beads, and the final purified product was resuspended in 15 µl of water. 1 µl of the 1:5 diluted PCR product was quantified using a Qubit2.0 Fluorometer and Qubit dsDNA HS Assay Kit. The Qubit measured PCR product was normalized to 0.2–0.4 ng/µl and analyzed using a High Sensitivity DNA Kit (Agilent, Cat No.5067-4626) on an Agilent Bioanalyzer. Smear analysis is performed on the region between 550–1,500 bp and the library yield is established. The concentration of the library was confirmed to be >4 nM.

ICELL8 Fiducial Mix

ICELL8 Fiducial Mix was dispensed into an unprinted ICELL8 150v Chip (Cat. No. 640013) in a predetermined pattern. The chip was imaged on the ICELL8 Imaging Station with the Micro-Manager software and analyzed using the 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 (Cat. Nos. 640164, 640165 & 640166), including the Second Diluent. Cells were dispensed into an unprinted ICELL8 Blank Chip (Cat. No. 640013). The chip was imaged on the ICELL8 Imaging System with the Micro-Manager software and analyzed using the 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.

640182

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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