Certificate of Analysis



ICELL8® cx TCR Chip

Catalog No. Amount Lot Number

640200 1 chip Specified on product label.

Description

The ICELL8 cx TCR Chip enables users to analyze T-cell receptor (TCR) diversity from single T cells using the ICELL8 cx Single-Cell System. Each well in the 5,184-nanowell chip contains a preprinted barcode, and 1,728 unique barcodes are present on each chip with each barcode printed in three locations on the chip. ICELL8 cx CellSelect® Software is used to detect wells bearing a single cell and direct nanowell-specific delivery of coupled SMART® technology RT-PCR reagents to only those wells on the ICELL8 cx TCR Chip that have been selected for further processing. Barcoded cDNA from the selected wells are amplified on-chip by PCR. The full-length barcoded amplicons are then pooled off-chip, and the purified cDNA is used as a template in the first TCR-specific PCR reaction. The product from the first TCR-specific PCR reaction is used as a template in a second TCR-specific PCR reaction to incorporate Illumina® indexed primers. Most importantly, the kit allows for efficient, cost-effective, and high-throughput single-cell capture of complete V(D)J variable regions of TCR transcripts.

Package Contents

• 1 x ICELL8 cx TCR Chip

Storage Conditions

• Store at -20°C.

Shelf Life

• Specified on product label.

Shipping Conditions

• Dry ice (-70°C)

Product Documents

Documents for our products are available for download at <u>takarabio.com/manuals</u> The following documents apply to this product:

- ICELL8 cx Human TCR a/b Profiling User Manual
- ICELL8 cx CellSelect Software User Manual

ICELL8 cx TCR Chip

Quality Control Data

Three chips from each lot were tested as follows:

Chip 1 was tested to ensure appropriate imaging quality performance: Fiducial mix and cells stained with Hoechst dye were dispensed into a subset of chip nanowells to align the chip and adjust the focus of the imaging system to the bottom of each well. The remaining wells were dispensed with 1X PBS and imaged in the DAPI channel using the ICELL8 cx instrument. Images of wells filled with 1X PBS were analyzed using CellSelect Software, and the number of wells for which the software detected a positive signal indicating the presence of a cell was counted and compared to the total number of wells assayed. The percentage of wells yielding images with a positive signal in the DAPI channel was confirmed to be <5%.

Chip 2 was tested to ensure barcodes are yielding appropriate reads per barcode: 5 pg of Jurkat Total RNA in 1X PBS was dispensed into the 1,728 wells of an ICELL8 cx TCR Chip containing unique barcodes. The RT-PCR reagents were then dispensed into these same wells. The chip was processed as per the ICELL8 cx Human TCR a/b Profiling User Manual. The concentration of the cDNA was confirmed to be ≥ 4 ng/ μ l with an average size > 600 bp. 500 pg of cDNA was used as template following two rounds of PCR as per user manual instructions. The concentration of the library was confirmed to be > 4 nM. The resulting library was pooled with the library from Chip 3. Pooled libraries were denatured and diluted to 13.5 pM with 5% PhiX and sequenced on a MiSeq® Sequencer using the 150-cycle (Read 1 = 75 cycles, Read 2 = 75 cycles, Index 1 = 8 cycles) MiSeq Reagent Kit v3 (Illumina, Cat No. MS-102-3001). Total reads per library were measured at > 8 x 10^6 with sufficient median diversity per barcode measured at > 90 normalized reads per barcode. > 95% of barcodes must be functional with > 0.2 normalized reads per barcode observed.

Chip 3 was tested to ensure barcode locations are as expected: 5 pg of Jurkat Total RNA in 1X PBS was dispensed into 180 specific wells of an ICELL8 cx TCR chip. These wells are unique and were expected to yield reads of expected barcodes only. The RT-PCR reagents were dispensed into these same wells. The chip was processed as per instructions in the ICELL8 cx Human TCR a/b Profiling User Manual. The concentration of the cDNA was confirmed to be ≥ 0.1 ng/µl with an average size >600 bp. 500 pg of cDNA was used as template following two rounds of PCR as per user manual instructions. The concentration of the library was confirmed to be >4 nM. The resulting library was pooled with the library from Chip 2. Pooled libraries were denatured and diluted to 13.5 pM with 2% PhiX and sequenced on a MiSeq Sequencer using the 150-cycle (Read 1 = 75 cycles, Read 2 = 75 cycles, Index 1 = 8 cycles) MiSeq Reagent Kit v3 (Illumina, Cat No. MS-102-3001). The library was then mixed with Chip-2-indexed libraries. Total reads per library were measured at >3 x 10^5 which was sufficient to determine that expected barcodes comprised the majority (>70%) of sequencing reads.

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

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640200

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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