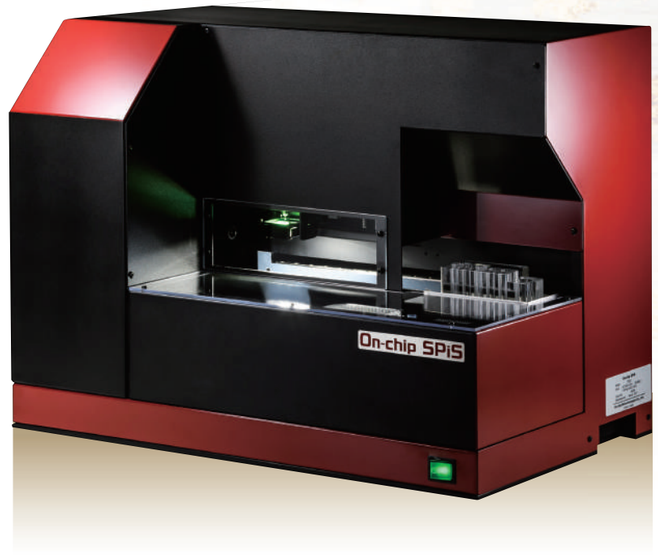
On-chip SPiS
Single Particle isolation System

Easy and reliable single cell and particle dispensing into well plates

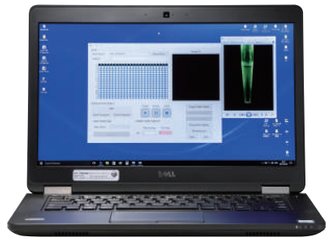


On-chip Biotechnologies Co., Ltd.

2-16-17 Naka-cho, Koganei-shi, Tokyo 184-0012, Japan
TEL.+81-42-385-0461 FAX.+81-42-385-0462

ONCHIP-03B-U003

On-chip SPiS : Single Particle isolation System



In recent years, single-cell analysis technology has been attracting attention. Single cell analysis requires accurate cell dispensing technology, but the lack of such technologies had been the bottleneck of research.
Limiting dilution widely used for cell cloning is a simple and low-cost method, but the single cell dispensing accuracy is low and repeated re-cloning operation is required. In contrast, dedicated single cell analysis instruments are expensive and inconvenient for general purposes, while the accuracy is not at a satisfactory level.
Therefore, On-chip Biotechnologies has developed On-chip SPiS in order to solve this problem. It is an easy-to-use instrument that allows dispensing of cells and large particles with single-cell/particle dispensing accuracy of over 90%.

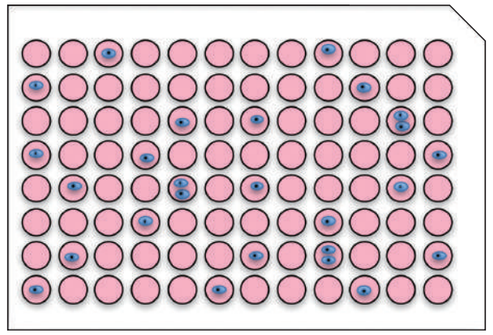
Feature of On-chip SPiS

Automatic, high-accuracy dispensing of cells into well plates

1. Easy and automatic operation
2. Disposable pipette tip-based dispensing system
3. Automatic dilution function + CCD camera image recognition
4. Dispensing of sample sized up to 200 μm
5. Reasonable price

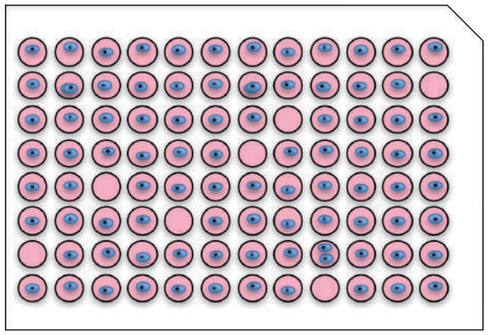
Conventional methods

(e.g., the limiting dilution method: 0.3 cells/well on average)



Accuracy in terms of one cell per well: ca. 21%

On-chip SPiS



Accuracy in terms of one cell per well: ca. 92%

Single cell dispensing workflow

① Automatic limiting dilution

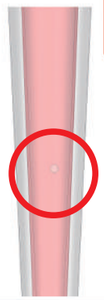
Automatic limiting dilution to a single cell per dispensed volume [a single cell/0.2 μL]

② Suction

Suction of a volume of liquid containing a single cell from the sample tube

③ Cell count confirmation

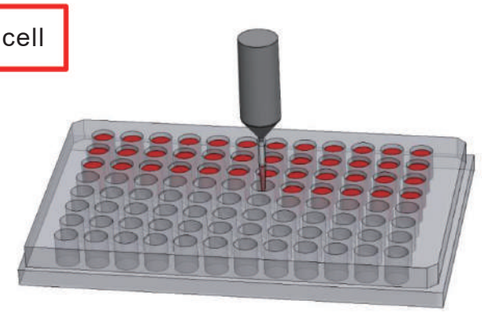
Measurement of the number of suctioned cells with a CCD camera



Only a single cell

No cell or two cells

④ Dispensing into a well



Reliable dispensing of one cell at a time

The process is retried until only a single cell is obtained.

Accuracy and analysis speed of single-cell dispensing

A549 cells were dispensed into 96 wells at 1 cell/well in 54 min. The number of wells with a single cell dispensed was 90, and cell proliferation was confirmed in 83 of them.

Dispensing time: 54 min/96 wells

Number of cells dispensed	Number of wells (%)	Number of wells with proliferated cells (%)
0	4 (4.2%)	-
1	90 (93.8%)	83 (92.2%)
2	2 (2.1%)	2 (100%)

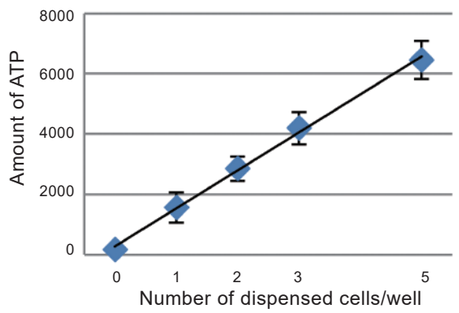
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	2	1	1	1	1	1	1	1	1	1	1
C	1	1	1	0	1	1	1	0	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	0	1
F	1	1	1	1	1	1	1	2	1	1	1	1
G	1	1	1	0	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

Each compartment represents a well, and the number shown in each compartment indicates the number of cells dispensed.

Confirmation of quantity-specified dispensing (ATP assay)

Quantity-specified dispensing

PC9 cells were dispensed into each well at 1, 2, 3, and 5 cells/well and the amount of ATP was measured, resulting in a good calibration curve in correlation with the number of cells.



Cell type: PC 9
The number of cells dispensed per well was specified to 1, 2, 3, or 5 cells/well.

Verification of dispensing accuracy based on the ATP amount

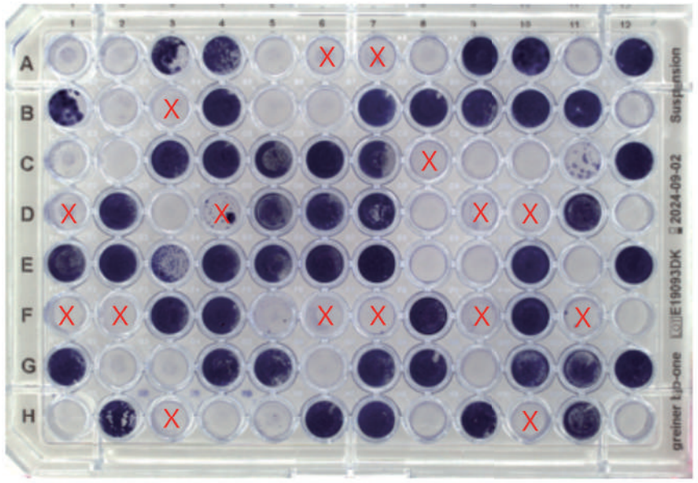
PC9 cells were dispensed into 96-well plates at 1 cell/well. Single cell dispensing accuracy of about 90% was determined by measuring the amount of ATP in each well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1560	1833	2893	710	1580	2213	986	893	860	2086	1020	726
B	886	840	793	1790	1276	1933	1796	766	1283	883	1970	810
C	1790	2703	3163	1676	2893	1840	1840	1640	2446	2720	2076	1670
D	2363	2173	1896	1713	2113	1706	3430	1583	2543	1400	2763	1783
E	1873	2223	2153	1710	1710	2693	1783	2030	1990	2163	3130	2753
F	2036	4021	2416	1186	1040	1110	1003	1233	1123	1243	2946	1846
G	950	1243	1756	2713	3303	2403	2150	1796	1666	2050	2483	2120
H	2356	3443	1700	2256	2076	4497	2756	2006	1950	2580	2483	2796

Dispensing accuracy of 90%
- A single cell was dispensed in each of 86 wells
- Two cells were dispensed in each of 10 wells

Single cell dispensing of knockout strains

On-chip SPiS is useful for post-genome editing screening. A poliovirus (PV) receptor knockout cell line was generated using CRISPR-Cas9. A single cell was dispensed into each well of a well plate and cultured, followed by viral infection. As a result, 49 clones that acquired PV resistance were successfully established.



PV resistance was obtained in 49 clones.
49 wells/96 wells = 51%

- Cells that acquired PV resistance
- Cells that didn't proliferate
- X Cells that failed to knockout the PV receptor and died of infection

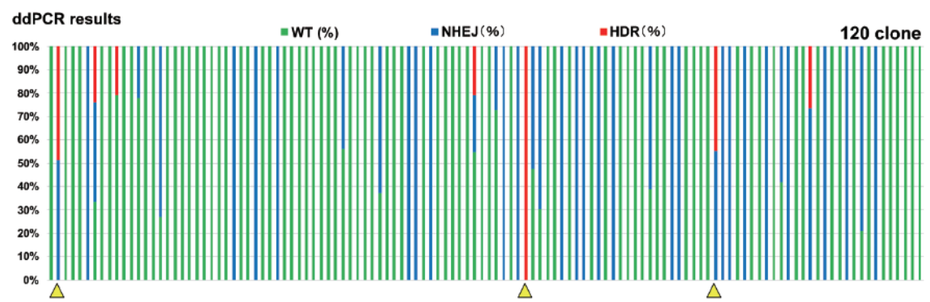
*Knockout of poliovirus receptor leaves cells uninfected and viable.

Collaboration with Dr. Koike, Tokyo Metropolitan Institute of Medical Science

HDR mutant clone selection

Transfected HEK293T cells were sorted by our microfluidic chip cell sorter (On-chip® Sort) to obtain positive strains, and single cells were dispensed by On-chip SPiS. As a result, genome-edited clones were efficiently obtained one by one. In addition, clones with homozygous mutations were efficiently obtained.

	Number
Dispensed cells	384
Obtained clones	120
Genome-editing occurredclones	41
Target HDR mutation clones (clones with homozygous HDR mutation)	7 (1)



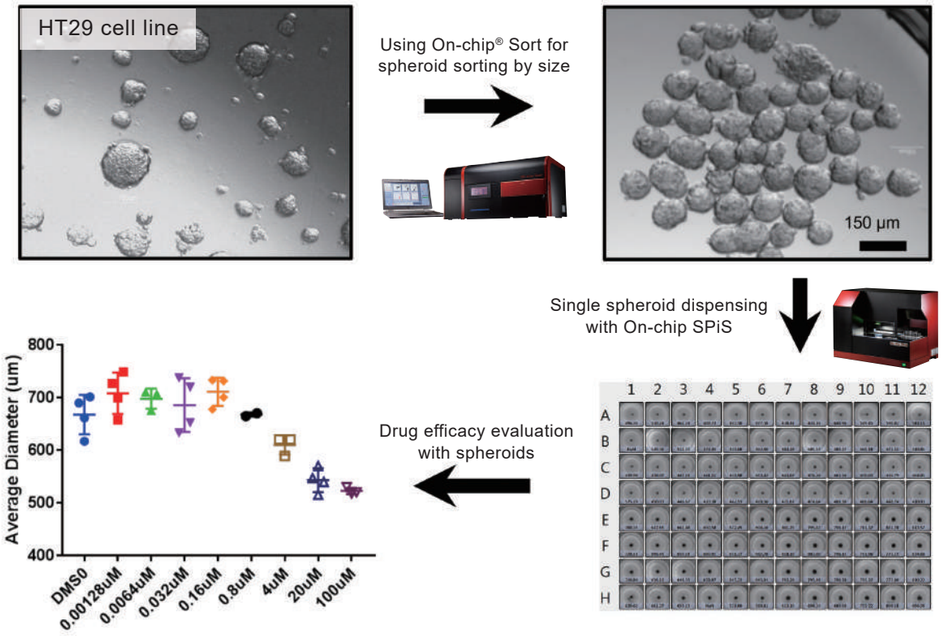
Clones with the target heterozygous or homozygous mutations(△).

*If the allele frequency was less than 20%, it was removed as zero.

Collaboration with Dr. Miyaoka, Tokyo Metropolitan Institute of Medical Science

Dispensing of spheroids of different sizes for drug evaluation

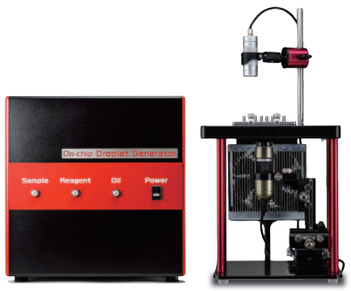
The use of spheroids (cell masses) which can reproduce the biological environment has attracted attention in the evaluation of drugs such as anti-cancer agents. Uniform sized spheroids are necessary for highly accurate evaluation.
Therefore, On-chip® Sort was used to select spheroids of a specific size and On-chip SPiS was used to dispense the collected spheroids on to a well plate for fast and accurate drug efficacy evaluation.



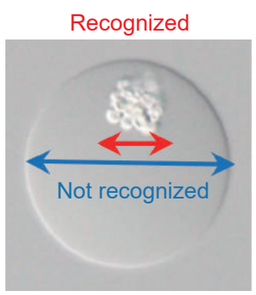
Collaboration with Mr. McClellan, Mitchell Cancer Institute

Dispensing of GMDs

On-chip SPiS can dispense not only cells and large particles, but also GMDs (microdroplets solidified as gels). Bacteria grown from a single cell can form microcolonies inside the GMDs, which can be recognized by the CCD camera in On-chip SPiS. The use of GMDs is useful in screening and isolating environmental microbes and generation of mutant libraries.



GMDs can be produced by our droplet generator (On-chip Droplet Generator)



Recognition of microcolonies grown in GMDs

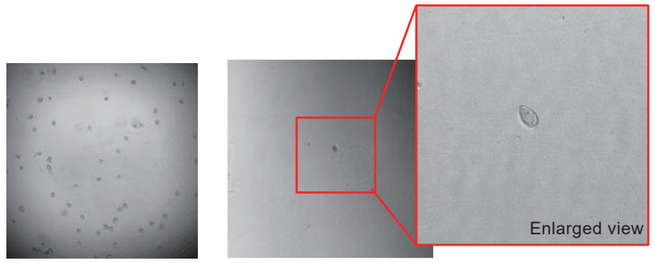
Application examples:

Screening and isolation of environmental microorganisms and mutant libraries

Dispensing and culture of swimming tetrahymena

Protozoan Tetrahymena were dispensed at 1 cell/well into a total of 180 wells and cultured for three days. Single cell dispensing was confirmed in 159 wells, 137 of which had cell proliferation. This result indicates that the system can dispense migrating cells with high accuracy.

Dispensing result



Before dispensing After dispensing

	Zero	One	Two	Three	Four or more	Total
Number of wells	19（10.6%）	159（88.3%）	1（0.6%）	1（0.6%）	0（0%）	180

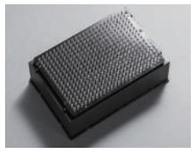
- Cell proliferation occurred : 137（86.2%）
- Cell proliferation didn't occur so much : 22（13.8%）

Collaboration with Dr. Nakano, Tsukuba University

Specification

Product name	On-chip SPiS
Product number	70001
Configuration	Main unit and control PC (Windows)
Main unit size	610 x 365 x 450 / W x D x H（mm）
Dispensing method	Disposable pipette tips
Recognition method	Image recognition by a camera with built-in 5-megapixel resolution CMO sensor
Tip volume	0.2 µL
Sample	Cells, pollen, protists, cell aggregates, beads, gel micro drops, etc.
Recognition size	10–200 µm (cultured cells are recognized)
Dispensing accuracy	≥ 90% (depending on sample conditions)
Biosafety	Installable in a biosafety cabinet
Damage to samples	Low damage to samples such as cells
Stirring method	Automatic stirring with the disposable pipette tip
Solution	Water, culture media, seawater, etc.
Processing speed	60 min/96 wells (changeable with setting conditions and sample conditions)
Supported plate	96-well plates, 384-well plates
Power input	AC 100-120V, 50/60Hz
Power consumption	1.0A typ（ACIN 100V）

Dispensing pipette tips for On-chip SPiS (sterile)
Usage: Used as pipette tips for dispensing.
Sterile pipette tips enable aseptic dispensing operations.
Part Number: 1007001 Part name: Chip-384S



For inquiries:
On-chip Biotechnologies Co., Ltd.
Phone: +81-42-385-0461 Fax: +81-42-385-0462
E-mail: info@on-chipbio.com Home page: https://www.on-chipbio.com