AXCELEAD
Drug Discovery Partners

Compound Screening Service using iPSC-derived Cells

Axcelead DDP and FUJIFILM Cellular Dynamics, Inc. support your drug discovery using iCell® Products

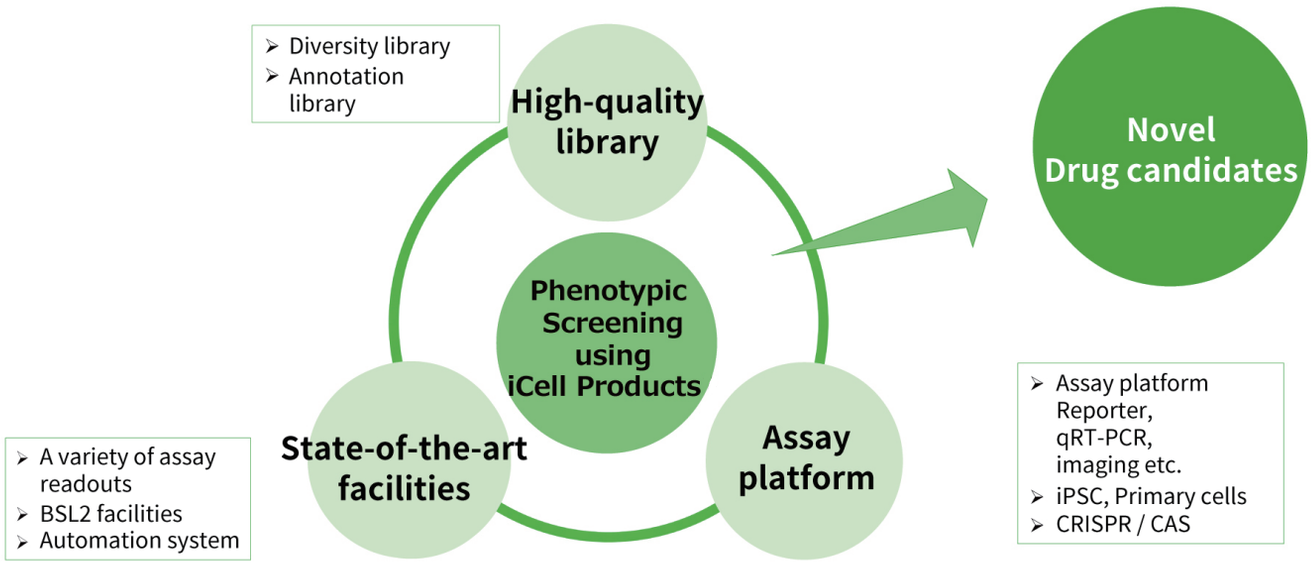
iPSC-derived cell types are useful physiologically relevant models of human diseases.
Recently, compounds discovered in phenotypic screening using iPSC-derived cells have been shown to be effective in clinical trials, thus making the use of iPSC-derived cells for drug screening an attractive approach.

Axcelead DDP supports your drug discovery from assay development through high-throughput screening using iPSC-derived cells from FUJIFILM Cellular Dynamics, Inc. (FCDI), a leading company of iPSC technologies.

FUJIFILM
Cellular Dynamics

Axcelead DDP Phenotypic Screening Services

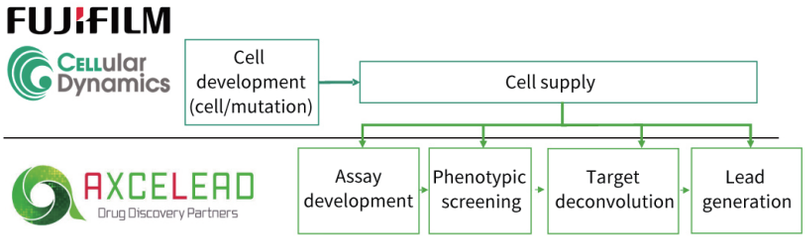
Utilizing the scientific experiences of Axcelead DDP and FCDI, we offer a wide selection of phenotypic screening services incorporating iCell®Products.



Our capabilities support you throughout your screening process:

- We can lead your drug discovery efforts using FCDI's iPSC technology and a wide range of screening experiences of Axcelead DDP.
- We can propose the best screening strategies according to your needs from assay development to HTS execution.
- Our vast library of pharma origin with high quality and diverse structures increases your chances in finding hit compounds.

An Axcelead researcher will be your point of contact for all services.



Case study

Screening for Phagocytosis Modulators using iCell® Microglia AD TREM2

Goal

To discover phagocytosis activators using iCell® Microglia AD TREM2 homozygous knockout (iCell Microglia TREM2 HO) cells.

Background

Mutations in the transmembrane protein, TREM2, has become a research focus because of its role in the binding and clearance of Aβ oligomers, thus making it a potential risk factor of Alzheimer's disease. Elucidation of the mechanism would be useful for development of therapies for Alzheimer's disease.

Assay Development

A biologically annotated compound library, consisting of about 3,400 compounds, was tested in a phagocytosis assay using iCell Microglia TREM2 HO to screen for phagocytosis activators. Compound concentration for the primary screening was set at 3 µM.

Assay flow

Day1
- Plate iCell Microglia into 384-well PDL-coated flat black/clear plate.

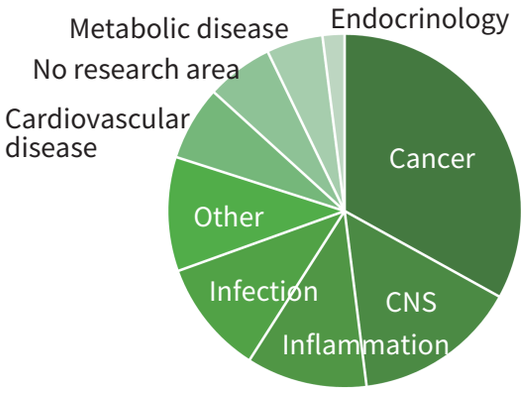
Day2-3
- Incubate cells with test compounds for 1 hour.
- Take images every 6 hours after the addition of fluorescence-labeled Aβ aggregates.

Day4
- Analyze phagocytosis activity at 48 hours using Incucyte® Zoom.

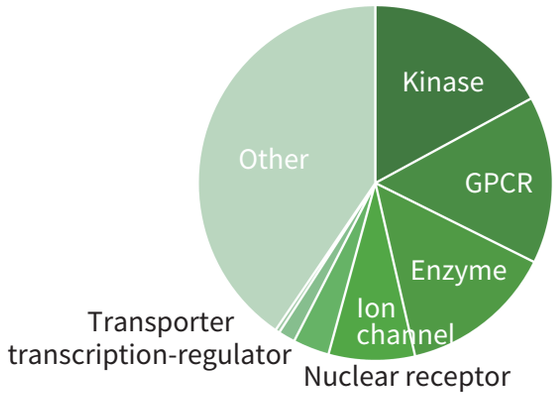
Biologically annotated compound library

Compound library consisting of small molecules with diverse biological and pharmacological activities.

Therapeutic classification of the library compounds evaluated



Biological classification of the library compounds evaluated

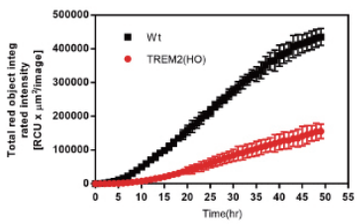


Assay Development Results

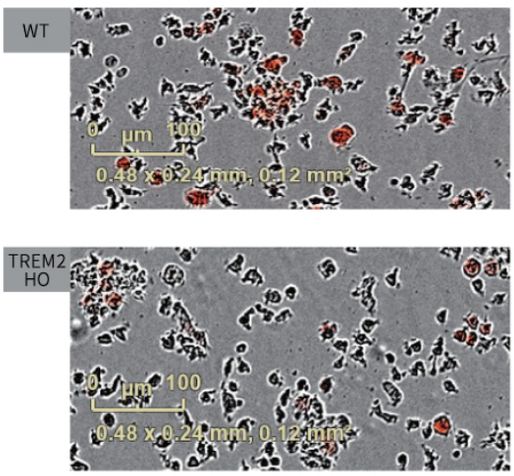
We optimized an assay to evaluate cellular uptake activity of fluorescent-labeled Aβ in a 384-well format using InCuCyte® ZOOM. Results showed Aβ uptake kinetics in iCell Microglia TREM2 HO was slower than that of iCell Microglia (wild type, WT).

Evaluation of Aβ uptake activity using iCell® Microglia AD TREM2 HO

IncuCyte® ZOOM



Time-course of microglial phagocytosis measured by IncuCyte® ZOOM. Phagocytosis activity of iCell Microglia TREM2 HO was about three times lower than that of iCell Microglia.



Representative images of iCell Microglia phagocytosis at 48hrs after Aβ addition; images were taken on the IncuCyte® ZOOM.

Screening Cascade Outline

Primary screening (c.a. 3400compounds)

▶Biologically annotated compounds, > 3400 cpds
▶3 μM, N=1
▶TREM2 mutant
▶Phagocytosis assay and cytotoxicity (CellTiter-Glo® Luminescent Cell Viability Assay)

Reproducibility test（350 compounds)

▶Positive compounds from primary screening
▶3 μM, N=1
▶TREM2 mutant
▶Phagocytosis assay and cytotoxicity (CellTiter-Glo® Luminescent Cell Viability Assay)

Dose response test (24 compounds)

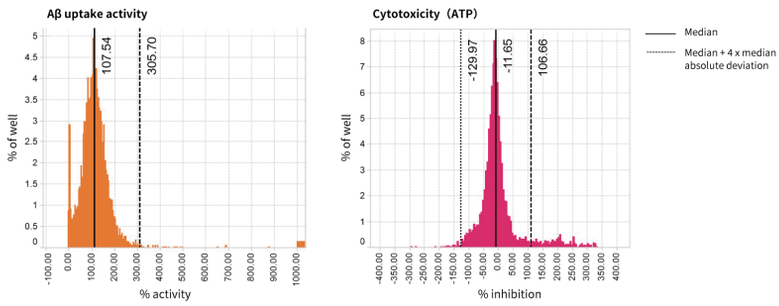
▶Selected compounds from reproducibility test
▶6 dose, N=2
▶TREM2 mutant and WT
▶Phagocytosis assay and cytotoxicity (CellTiter-Glo® Luminescent Cell Viability Assay)

Hit compounds

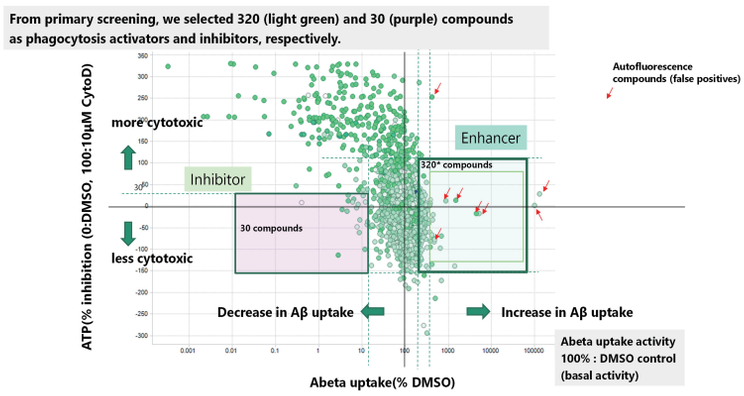
Primary Screen Results

After the primary screening step, false-positive compounds (e.g.Auto-fluorescence, cell toxicity) were removed. Based on the activity distribution from the primary screening, positive compounds were selected for a following screening cascade, reproducibility assay and concentration-response assay.

Histograms for tested compounds in the primary screening



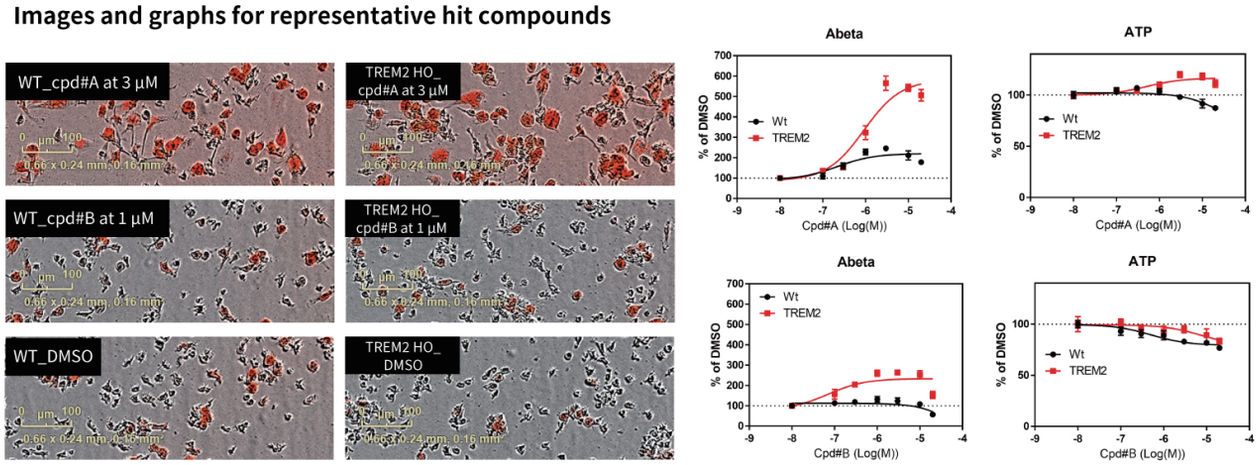
Correlation between Aβ uptake activity and cytotoxicity in the primary screening



Dose Response Results

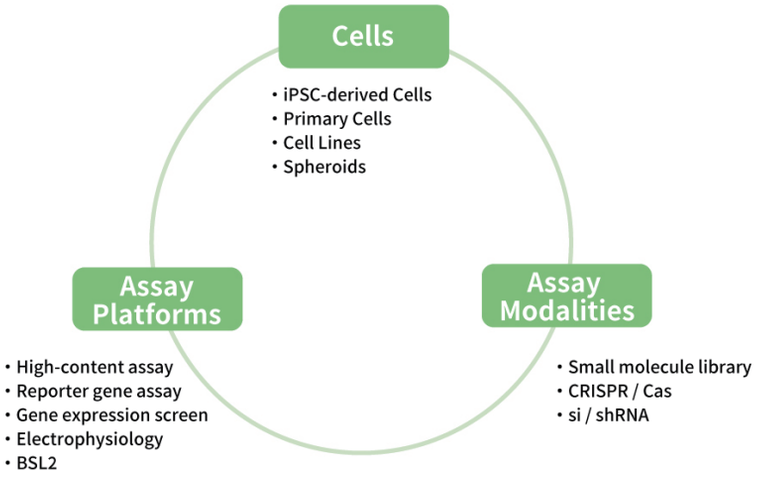
We successfully identified several phagocytosis activators. These compounds enhanced phagocytosis activity without severe cytotoxicity.
Representative compounds, A and B, increased the phagocytosis activity in iCell Microglia TREM2 HO comparable to levels of iCell Microglia. Compound A enhanced phagocytosis activity in both iCell Microglia and iCell Microglia TREM2 HO, while compound B increased it only in iCell Microglia TREM2 HO, suggesting that these compounds modulated phagocytosis activity through different mode-of–actions.

Images and graphs for representative hit compounds



A Variety of Assays and Experiences in Phenotypic Screening

Our vast experience in phenotypic screening combined with our diverse compound libraries provides high-quality phenotypic screening services.



HORNET HTS-10CB
(FUJIFILM Wako)

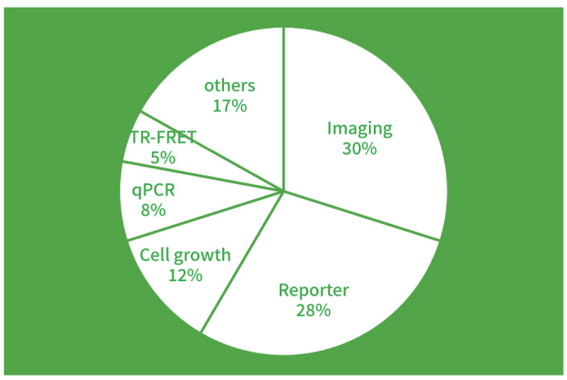
QuantiStudio 12K Flex
Realtime PCR
(ThermoFisher)

IN Cell Analyzer 6000
(Molecular Devices)

Syncropatch 384
(Nanion)

Track Record

Assay methods used for ～70 phenotypic screens at Axcelead DDP



Assay method	Typical size of library
Imaging	100K compounds
Reporter gene assay	- 400K compounds
Cell growth	- 400K compounds
qRT-PCR	30 K compounds
TR-FRET	100K compounds
ELISA	100K compounds
POI-HiBit screening (for degrader screening)	100K compounds

Target Deconvolution

Axcelead's researchers from various fields collaborate to provide a one-stop solution, including target deconvolution.

Target deconvolution

- Phenotypic screening
- Target-unknown drugs

→ **Annotation analysis**

Chemical proteomics | **Finger printing**

→

- Target based drug discovery
- Drug repositioning

Axcelead Drug Discovery Partners, Inc.

26-1, Muraoka-Higashi 2-chome
Fujisawa, Kanagawa 251-0012, Japan
www.axcelead.com
contact@axcelead.com

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