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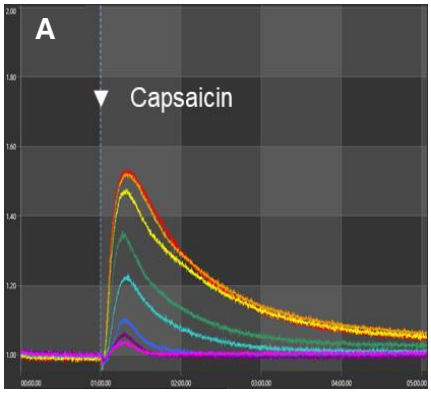
Calcium Flux Assay with iCell® Sensory Neurons

iCell Lab Note

Introduction.

Calcium flux is an essential assay readout for utilizing and evaluating human induced pluripotent stem cell (iPSC)-derived sensory neurons for pain therapeutic drug discovery. This technique measures the change of global intracellular calcium in response to the activation of ion channels and GPCRs. Using calcium flux, iCell Sensory Neurons show responsiveness across a range of known sensory neuron agonists, including capsaicin, menthol, and ATP; each of which stimulate specific nociceptive receptors. This iCell® Lab Note provides technical guidance and representative data for the culture and assay of iCell Sensory Neurons in 384-well format using a Ca^{2+} indicator dye and capsaicin as the control agonist. Importantly, this assay is amenable to high throughput compound testing and data from a TRPV1 antagonist screen is presented.



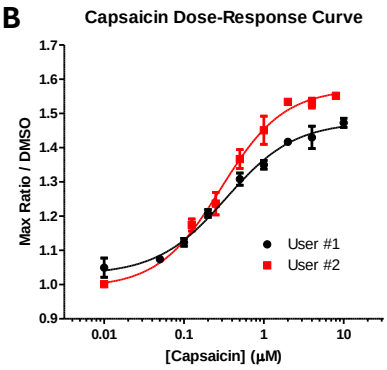


Figure 1. Capsaicin Dose Response. iCell Sensory Neurons were plated in a 384-well format and maintained in Complete iCell Sensory Neurons Medium until Day 21. **A)** Capsaicin (0→8 µM) was used to trigger Ca^{2+} flux on the FDSS/µCell (Hamamatsu). **B)** Dose-response curves were generated consistently between operators. ***Note:*** *Media and supplements provided with the cells are critical for a robust capsaicin response on the day of assay.*

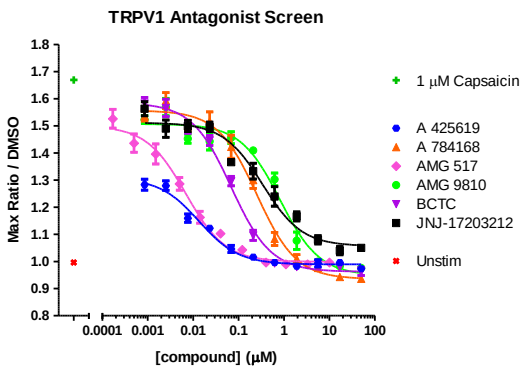


Figure 2. Compound Profiling. Following the workflow outlined in this iCell Lab Note, calcium flux in iCell Sensory Neurons was pharmacologically modulated with known TRPV1 inhibitors. Compounds were tested in triplicate using an 11-point dose-response in the presence of 1 µM Capsaicin. ***Note:*** *TRPV1 antagonists were from Tocris.*

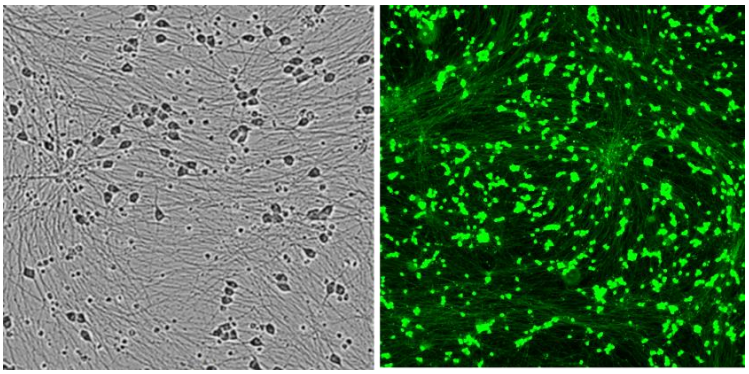
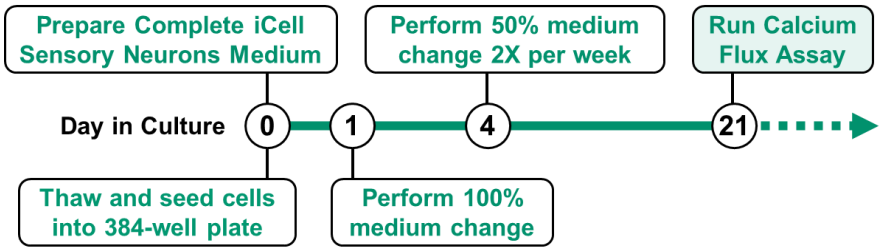


Figure 3. Morphology and Dye Loading Efficiency. Phase contrast (left) and green fluorescent (right) images of iCell Sensory Neurons loaded with calcium indicator dye on Day 21 in culture. Cells were cultured on a PDL/laminin 511-coated plate at 14,000 cells/well. Images were acquired on an Incucyte SX5 live cell imager with a 10X objective.

Cellular Dynamics

Assay Workflow Schematic.



Methods.

Prepare Complete iCell Sensory Neurons Medium and thaw cells according to the User's Guide.

- Day 0: Coat 384-well plate with PDL solution (50 µg/ml for 1 h at 37°C), wash 3X with sterile water, then coat with Laminin iMatrix-511 (2.5 µg/ml; 1 h; 37°C).
  - Use viable number of cells/vial on COA.
  - Recommended cell density is 14K-19K per well.
- Day 1: Perform a 100% media change.
- Days 4-20: Change 50% media 2X per week until day of assay.
- Day 21 or later: Prepare assay buffer with 1X HBSS (-/-), 2 mM $CaCl_2$ and 20 mM HEPES.
  - Remove culture media and replace with Calcium 6 dye (resuspended in assay buffer); incubate for ≥2 h at 37°C.
  - During dye loading incubation, prepare capsaicin doses in assay buffer (e.g., 5X final concentration) or pre-treat cells with antagonists for 30-60 min, if desired.
- Proceed with calcium flux assay.

Table 1. Materials Needed.

Product	Vendor	Cat. #
iCell Sensory Neurons Kit, 01279 [1]	FCDI	R1250
▪ iCell Sensory Neurons Base Medium	(incl. in kit)	M1052
▪ iCell Sensory Neurons Supplement (100X)	(incl. in kit)	M1053
Poly-D-Lysine (PDL) Solution [2]	GIBCO	A3890401
Recombinant Laminin iMatrix-511 Silk [3]	AMSBIO	AMS.892021
384-well Cell Culture Plate [3]	Greiner	781091
FLIPR Calcium 6 Assay Kit [3]	Molecular Devices	R8190
Capsaicin [3]	Sigma	PHR1450
JNJ-17203212 [4]	Tocris	3361

[1] iCell Sensory Neurons from Donor 21527 (R1252) are also available.
[2] Manual coating of cell culture plate with PDL solution is recommended over the use of PDL pre-coated plates for this application.
[3] Alternative materials, plates, calcium dyes, and agonists from other sources may be compatible in this assay but have not been fully tested.
[4] Different TRPV1 antagonists have been profiled. See Figure 2 or inquire for more info.

Scan here to download the iCell Sensory Neurons User's Guide.

Summary.

This iCell Lab Note provides the basic instructions for measuring calcium flux in iCell Sensory Neurons following stimulation with Capsaicin. This approach can also be used to test different sensory agonists, including ATP (P2RX3), menthol (TRPM8), or Yoda1 (PIEZO1). Alternatively, other fluorescent ion indicators (i.e., sodium dyes) may be used with ion channel-specific compounds to expand the biophysical characterization of iCell Sensory Neurons.

Highlights.

- iCell Sensory Neurons respond to Capsaicin by Day 21 in calcium flux assay.
- High-throughput 384-well assay enables compound screening in dose-response format.
- Calcium flux protocol can be used with alternate sensory agonists, imaging platforms, or fluorescent ion indicator dyes.

Contact **Technical Support** (FCDI-Support@fujifilm.com) for more protocol details and supportive data.

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