FUJIFILM
Cellular Dynamics

iCell® Sensory Neurons
User's Guide

Document ID: X1051

Restrictions and Liabilities

This document is provided “as is.” FUJIFILM Cellular Dynamics, Inc. (FCDI), assumes no responsibility for any typographical, technical, or other inaccuracies in this document. FCDI reserves the right to periodically change information that is contained in this document; however, FCDI makes no commitment to provide any such changes, updates, enhancements, or other additions to this document to you in a timely manner or at all.

OTHER THAN THE EXPRESS LIMITED WARRANTY SET FORTH IN WWW.FUJIFILMCDI.COM/TERMS-AND-CONDITIONS/, FCDI MAKES NO, AND DISCLAIMS ALL, REPRESENTATIONS, WARRANTIES, CONDITIONS OR COVENANTS, EXPRESS OR IMPLIED, WITH RESPECT TO ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN, INCLUDING BUT NOT LIMITED TO, ANY EXPRESS OR IMPLIED WARRANTIES OR CONDITIONS OF FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, MERCHANTABILITY, DURABILITY, TITLE, OR RELATED TO THE PERFORMANCE OR NON-PERFORMANCE OF ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN).

This document might contain references to third-party sources of information, hardware or software, products or services and/or third-party websites (collectively the “Third-Party Information”). FCDI does not control, and is not responsible for, any Third-Party Information, including, without limitation the content, accuracy, copyright compliance, compatibility, performance, trustworthiness, legality, decency, links, or any other aspect of Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement by FCDI of the Third-Party Information or the third party in any way.

FCDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Sensory Neurons as described herein. The only warranties provided to you are included in the Limited Warranty set forth in www.fujifilmcdi.com/terms-and-conditions/. You assume all risk in connection with your use of iCell Sensory Neurons.

Conditions of Use

iCell Sensory Neurons are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. See www.fujifilmcdi.com/terms-and-conditions/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.

Trademarks

iCell and MyCell are registered trademarks, and the Cellular Dynamics logo are trademarks of FUJIFILM Cellular Dynamics, Inc. and may not be used without the express written permission of FUJIFILM Cellular Dynamics, Inc.

All other brands, product names, company names, trademarks, and service marks are the properties of their respective owners.

Origin

iCell Sensory Neurons are manufactured in the United States of America.

Copyright Notice

Revision History

Document ID: X1051
Version 2.0: June 2024

Table of Contents

Before You Begin

- Immediately transfer the frozen vials of iCell Sensory Neurons to liquid nitrogen storage.
- Read this entire User's Guide before handling or using iCell® Sensory Neurons.
- iCell Sensory Neurons are FOR RESEARCH USE ONLY and NOT FOR THERAPEUTIC USE. See www.fujifilmcdi.com/terms-and-conditions/ for USE RESTRICTIONS applicable to the cells and other terms and conditions related to the cells and their use.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Sensory Neurons are frozen, is available online at www.fujifilmcdi.com/product-literature/ or on request from FUJIFILM Cellular Dynamics. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Sensory Neurons.

Chapter 1. Introduction

iCell Sensory Neurons from FUJIFILM Cellular Dynamics, Inc. (FCDI), are a highly pure population of human sensory neurons derived from induced pluripotent stem (iPS) cells. These neurons are generated using directed differentiation protocols that result in a biologically relevant human sensory neuron population. iCell Sensory Neurons grow neurites, maintain health over multiple weeks in culture (Figure 1), and express characteristic sensorineural markers (Figure 2). They also express expected sensory receptors (TRPV1, TRPM8, P2RX3) and channels (NaV1.7, Nav1.8) and exhibit expected physiological responses to sensory channel agonists (Figure 3).

In vitro models of sensory neurons are valuable for investigating next-generation analgesics, chemotherapeutic compounds, and understanding the molecular mechanisms of pain and neuropathy. iCell Sensory Neurons provide a reliable source of human-relevant iPSC-derived sensory cells for use in high throughput peripheral neuropathy and pain drug discovery programs. They also offer a consistent off-the-shelf source of human sensory neurons to support compound validation, efficacy, and safety testing (Figure 4).

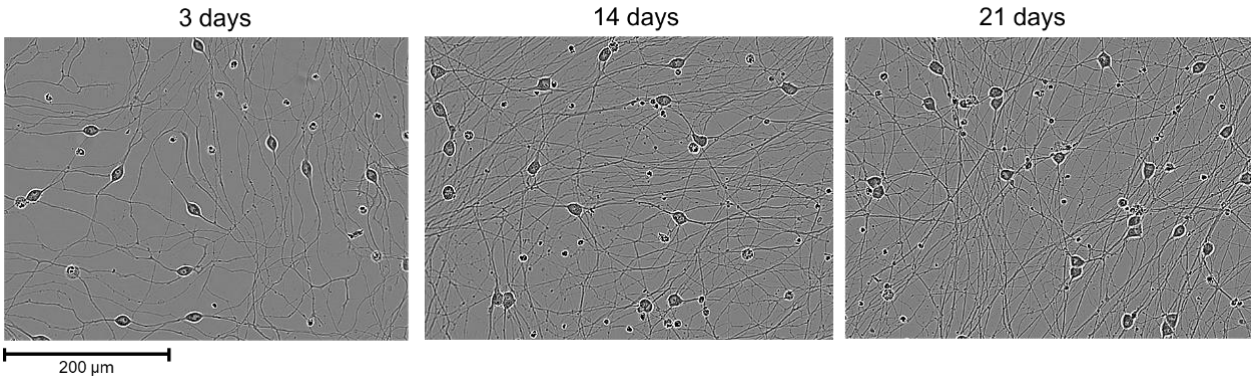


Figure 1: iCell Sensory Neurons Maturation

iCell Sensory Neurons, 01279 were plated on poly-ornithine and laminin-coated glass coverslips at 2.0x10^4 cells/cm^2 and cultured using Complete Sensory Neurons Medium for 21 days. Representative brightfield images show healthy cell soma and extension and elaboration of neurite networks.

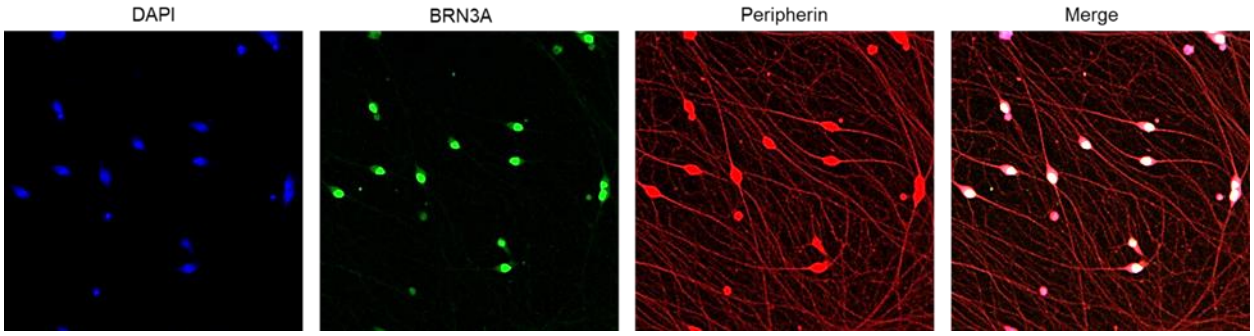


Figure 2: iCell Sensory Neurons Express Sensorineural Markers

iCell Sensory Neurons, 01279 were cultured at 1.0x10^4 cells/well in a 96-well plate for 7 days prior to fixation and staining for the sensory neuron transcription factor, BRN3A (green), and the peripheral neurofilament, Peripherin (red). The merged image on the right shows colocalization of the two sensorineural markers along with DAPI as a nuclei marker.

Components Supplied by FUJIFILM Cellular Dynamics

Item	Catalog Number
iCell Sensory Neurons Kit, 01279 • iCell Sensory Neurons, 01279[1] • iCell Sensory Neurons Base Medium[1] • iCell Sensory Neurons Supplement (100X)[1] • iCell Sensory Neurons User's Guide	R1250 (≥ 6.0 x 10^6 viable cells) or R1251 (≥ 1.0 x 10^6 viable cells) • C1259 (≥ 6.0 x 10^6 viable cells) or C1260 (≥ 1.0 x 10^6 viable cells) • M1052 (100 ml) • M1053 (1 ml) • X1051
iCell Sensory Neurons Kit, 21527 • iCell Sensory Neurons, 21527[1] • iCell Sensory Neurons Base Medium[1] • iCell Sensory Neurons Supplement (100X)[1] • iCell Sensory Neurons User's Guide	R1252 (≥ 6.0 x 10^6 viable cells) or R1253 (≥ 1.0 x 10^6 viable cells) • C1261 (≥ 6.0 x 10^6 viable cells) or C1262 (≥ 1.0 x 10^6 viable cells) • M1052 (100 ml) • M1053 (1 ml) • X1051
iCell Sensory Neurons Media Kit • iCell Sensory Neurons Base Medium[1] • iCell Sensory Neurons Supplement (100X)[1]	R1259 • M1052 (100 ml) • M1053 (1 ml)
Certificate of Analysis[2]	
Certificate of Origin If required for shipping purposes	

[1]Safety Data Sheet and User's Guide available online: www.fujifilmcdi.com/product-literature/

[2]Available online: www.fujifilmcdi.com/coa-lookup/

Required Equipment and Consumables

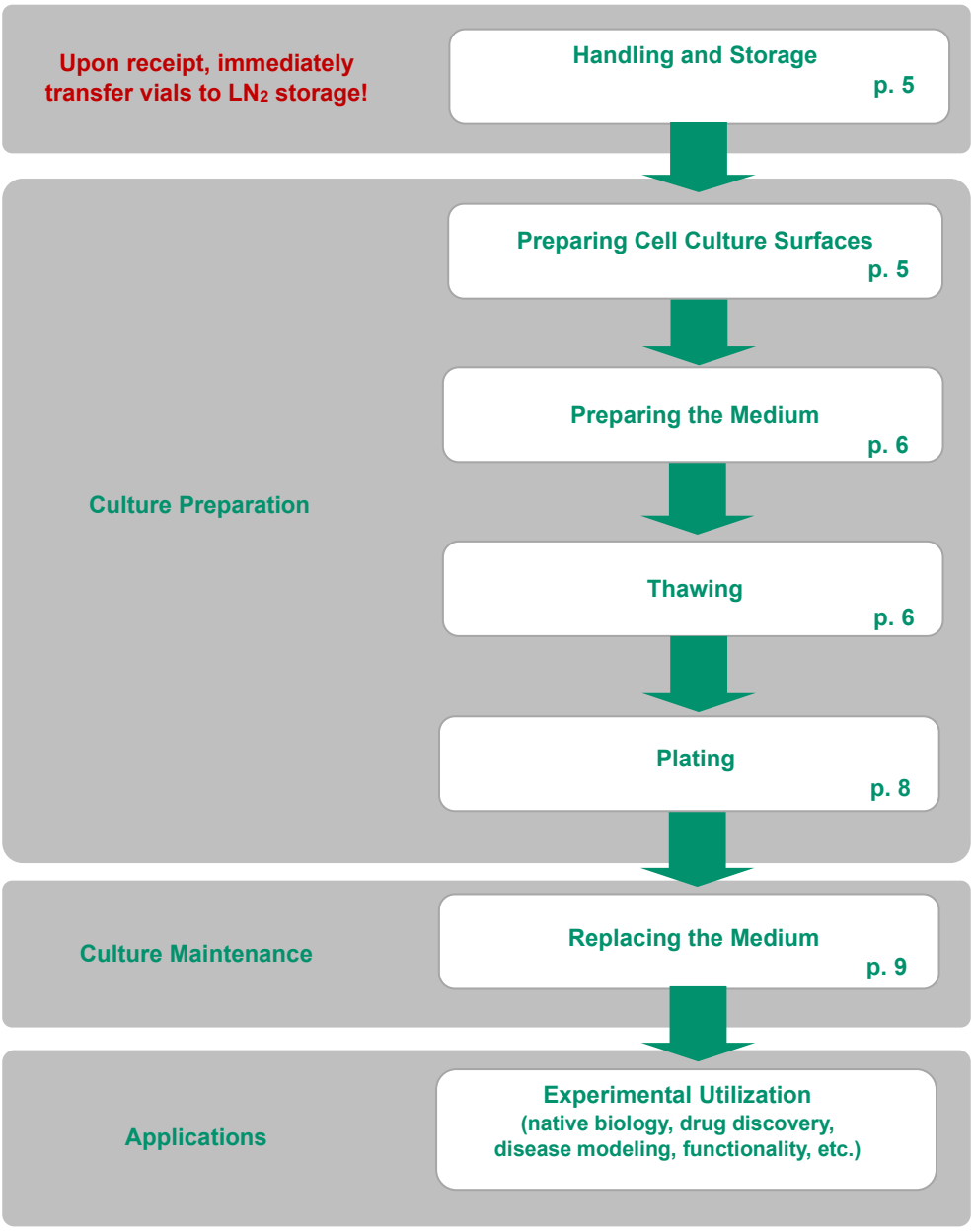
Item	Vendor(s)	Catalog Number(s)
Equipment		
37°C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
0.2 µm Sterile Filter Unit	Multiple Vendors	
6-well Flat-bottom Plate, TC-treated, Costar	Multiple Vendors	
24-well Flat-bottom Plate, TC-treated, Costar	Multiple Vendors	
96-well Flat-bottom Plate, TC-treated, Falcon	Multiple Vendors	
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)	Multiple Vendors	
Geltrex Basement Membrane Matrix (Geltrex Matrix)	Thermo Fisher Scientific	A15696-01
Serological Pipettes, multiple sizes	Multiple Vendors	

Technical Support, Knowledge Base, and Training

FCDI's Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. Our web-based Knowledge Base provides solutions for iCell related questions about plating and media, cell culture, general assay methods, and more. In addition, in-lab training may be available upon request.

Telephone	(877) 320-6688 (US toll-free) / (608) 310-5100 x3 Monday - Friday, 8:30 am - 5:00 pm US Central Time
Email	fcdi-support@fujifilm.com
Knowledge Base	www.fujifilmcdi.com/knowledge-base/

Workflow Diagram



Chapter 2. Handling and Storage

Handling iCell Sensory Neurons

iCell Sensory Neurons are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Sensory Neurons to the vapor phase of a liquid nitrogen storage dewar. FCDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.

It is critical to maintain cryopreserved iCell Sensory Neurons at a stable temperature. Minimize exposure of cryopreserved iCell Sensory Neurons to ambient temperature when transferring vials to liquid nitrogen storage.

Handling iCell Sensory Neurons Medium and Supplement

iCell Sensory Neurons are shipped with two (2) additional components: iCell Sensory Neurons Base Medium and iCell Sensory Neurons Supplement (100X). These medium components are shipped frozen on dry ice. Upon receipt, store each component at -20°C until use.

Chapter 3. Preparing Cell Culture Surfaces

iCell Sensory Neurons will maintain function on a freshly prepared plate with a coating of Geltrex Basement Membrane Matrix solution, which are recommended to promote iCell Sensory Neurons attachment, long term viability, and function.

Cell culture surfaces can influence adherence, morphology and function of iCell Sensory Neurons. Alternative substrates to Geltrex include iMatrix-511 Recombinant Laminin E8 Fragments or a combination of a base layer of poly-L-ornithine (PLO) with a top coating of laminin. Contact Technical Support for additional substrates amenable to the iCell Sensory Neurons.

Prepare plating surfaces before thawing iCell Sensory Neurons.

1. Select the cell culture vessel appropriate for your experimental use. Use the volumes specified in the table below in the following coating procedure. Scale volumes appropriately for other vessel formats.

   Note: *Concentrated basement membrane-derived extracellular matrix substrates, including Thermo Fisher Cat. No. A1413202, can also be used at a concentration of 120-180 µg/ml and diluted with ice cold DMEM/F12, following the manufacturer's instructions.*

Culture Vessel	Volume of Geltrex Solution (ml)
6-well Cell Culture Plate	1
24-well Cell Culture Plate	0.6
96-well Cell Culture Plate	0.1

Table 1: Summary of Useful Volumes
*All volumes are **per well**.*

It is critical to maintain the Geltrex reagent on ice. Geltrex will begin to gel if kept above 15°C for an extended period of time. Consult the manufacturer's instructions for details on proper handling of Geltrex.

2. Add appropriate volume of the Geltrex solution to each well and incubate the vessel(s) in a 37°C cell culture incubator for at least 1 hour.

   Note: *Alternatively, add the Geltrex solution to each well, wrap the vessel(s) in plastic paraffin film, and store overnight at 4°C. Equilibrate the vessel(s) in a 37°C cell culture incubator before use.*

3. Aspirate the Geltrex solution immediately before the addition of the cell suspension.

Do not allow the Geltrex-coated surface to dry. Drying of the culture surface can lead to cell clumping and migration.

Chapter 4. Preparing the Medium

The Complete Sensory Neurons Medium is comprised of 2 components: iCell Sensory Neurons Base Medium and iCell Sensory Neurons Supplement (100X). The Complete Sensory Neurons Medium is serum-free, antibiotic-free, and has been specially formulated to maximize cell viability, recovery at thaw, and maturation of function of cultured iCell Sensory Neurons. Thaw and store the media as follows:

Note: *One bottle of iCell Sensory Neurons Base Medium and iCell Sensory Neurons Supplement (100X) will provide enough Complete Sensory Neurons Medium to culture up to one 96-well plate of iCell Sensory Neurons for up to 3 weeks.*

1. Thaw iCell Sensory Neurons Base Medium and iCell Sensory Neurons Supplement (100X) overnight at 4°C.

Do not thaw the medium or supplement in a 37°C water bath.

2. Spray medium component with 70% ethanol and place in a biological safety cabinet.
3. Using sterile technique, add the entire contents of the iCell Sensory Neurons Supplement (100X) to the iCell Sensory Neurons Base Medium bottle (~100 ml) to make the Complete Sensory Neurons Medium.
4. Store the Complete Sensory Neurons Medium at 4°C for up to 3 weeks.

Chapter 5. Thawing iCell Sensory Neurons

Store iCell Sensory Neurons in liquid nitrogen (vapor phase) until immediately before thawing to ensure maximal recovery and performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Sensory Neurons viability and performance.

Note: *Thaw no more than 2 vials of iCell Sensory Neurons at one time.*

1. Equilibrate the appropriate amount of the Complete Sensory Neurons Medium to room temperature before thawing iCell Sensory Neurons.
2. Remove the iCell Sensory Neurons cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place cryovials on dry ice for up to 10 minutes before thawing.*

3. Immerse the cryovial in a 37°C water bath for 3 minutes (avoid submerging the cap), holding the tube stationary and without swirling. Use of a floating microcentrifuge tube rack is recommended.

Precise timing is critical to maximize viable cell recovery.

4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iCell Sensory Neurons cryovial contents to a sterile 50 ml centrifuge tube using a 1 ml pipettor.

   Note: *Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase neuron viability.*

Avoid repeated pipetting of the thawed iCell Sensory Neurons cell suspension.

6. Rinse the empty iCell Sensory Neurons cryovial with 1 ml of Complete Sensory Neurons Medium to recover any residual cells from the vial.
7. Transfer the 1 ml of the Complete Sensory Neurons Medium rinse from the cryovial drop-wise (~1 drop/sec) to the 50 ml centrifuge tube containing the iCell Sensory Neurons cell suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.

Drop-wise addition of the medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and recovery.

8. Slowly add 8 ml of the Complete Sensory Neurons Medium to the 50 ml centrifuge tube drop-wise (~1 drops/sec) while gently swirling.

It is critical to add the 8 ml of the Complete Sensory Neurons Medium slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.

9. Centrifuge the cell suspension at 400 x g for 5 minutes at room temperature.
10. Carefully aspirate the supernatant, gently resuspend the cell pellet in an appropriate volume (e.g., 3 ml) of Complete Sensory Neurons Medium by pipetting up and down 2 - 3 times.

Chapter 6. Plating iCell Sensory Neurons

iCell Sensory Neurons can be plated at a range of cell densities (0.3×10^5 – 1.875×10^5 cells/cm^2). The recommended plating density for iCell Sensory Neurons is 0.625×10^5 viable cells/cm^2 (or 20,000 cells/well for a 96-well plate). Table 2 provides the desired cell number and plating volume for several common cell culture plates when plating at a density of 0.625×10^5 viable cells/cm^2.

1. Obtain the number of viable cells/vial and viability from the Certificate of Analysis.
2. Calculate the final volume of Complete Sensory Neurons Medium needed to obtain the desired cell plating density using the number of viable cells/vial from the Certificate of Analysis. See Table 2 below for examples.
3. Dilute the cell suspension with room temperature Complete Sensory Neurons Medium to obtain a desired cell plating density.
4. Aspirate the Geltrex solution from the pre-coated cell culture plates.

> *Do not allow the coated surface to dry prior to the addition of cells.*

5. Dispense the cell suspension into the appropriate cell culture vessel(s).
6. Culture iCell Sensory Neurons in a cell culture incubator at 37°C, 5% CO_2 for 24 hours.

Expected Cell Density

iCell Sensory Neurons can be plated at various densities to accommodate different applications. However, 0.625×10^5 viable cells/cm^2 is the recommended density range for most applications. The following table provides the desired cell number and plating volume for several common cell culture vessels. Contact Technical Support for cell densities utilized for specific assays.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (0.625×10^5 cells/cm^2)
6-well Cell Culture Plate	9.6	2	600,000
24-well Cell Culture Plate	1.9	0.6	120,000
96-well Cell Culture Plate	0.32	0.1	20,000

Table 2: Summary of Recommended Volumes and Measures

*All volumes and measures are **per well**.*

Chapter 7. Maintaining iCell Sensory Neurons

1. Immediately before use, equilibrate Complete Sensory Neurons Medium to room temperature.
2. 24 hours post-plating, aspirate 100% of the medium from each well and replace with same volume of fresh Complete Sensory Neurons Medium as specified on Table 2.

> *It is recommended to change the medium a few wells at a time to minimize the time without medium for the cells.*

3. Every 3 to 4 days, replace 50% of the medium with the same volume of fresh Complete Sensory Neurons Medium.
4. Culture iCell Sensory Neurons in a cell culture incubator at 37°C, 5% CO_2.

Chapter 8. Representative Data

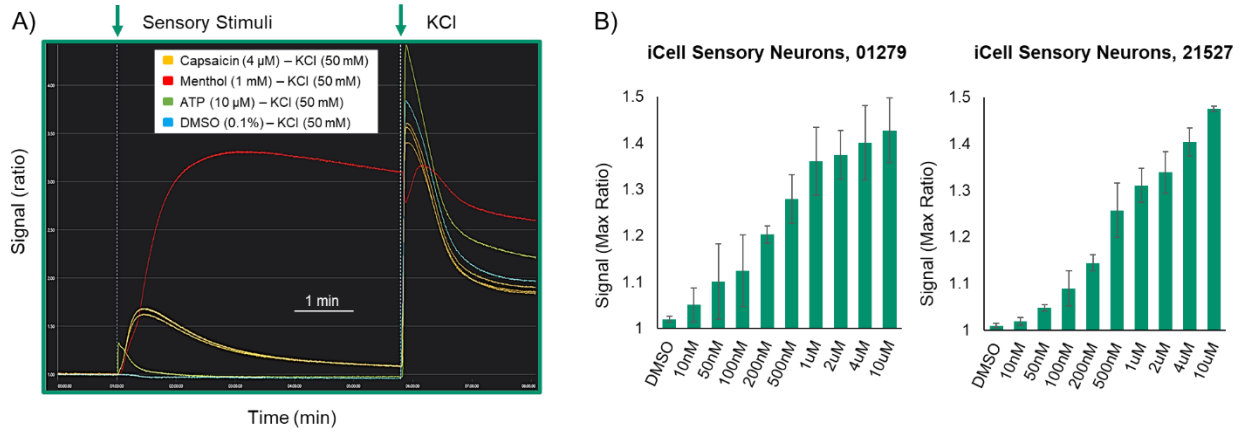


Figure 3: iCell Sensory Neurons Respond to Sensory Stimuli

iCell Sensory Neurons were cultured at 6x10^5 cell/well of a 96-well plate for 21 days and evaluated for responses to sensory stimuli via fluorescent calcium indicator assay. ***A)*** *Representative calcium influx traces in iCell Sensory Neurons, 01279 in response to Capsaicin (TRPV1 agonist), Menthol (TRPM8 agonist), and ATP (P2RX3 agonist). DMSO (0.1%) was used as the negative control. Potassium chloride (KCl) was added as a positive control after each stimulus (second horizontal blue line).* ***B)*** *Capsaicin dose-responses for 01279 and 21527 iCell Sensory Neurons. Responses to capsaicin are observed with low concentrations of capsaicin (10 – 50 nM).*

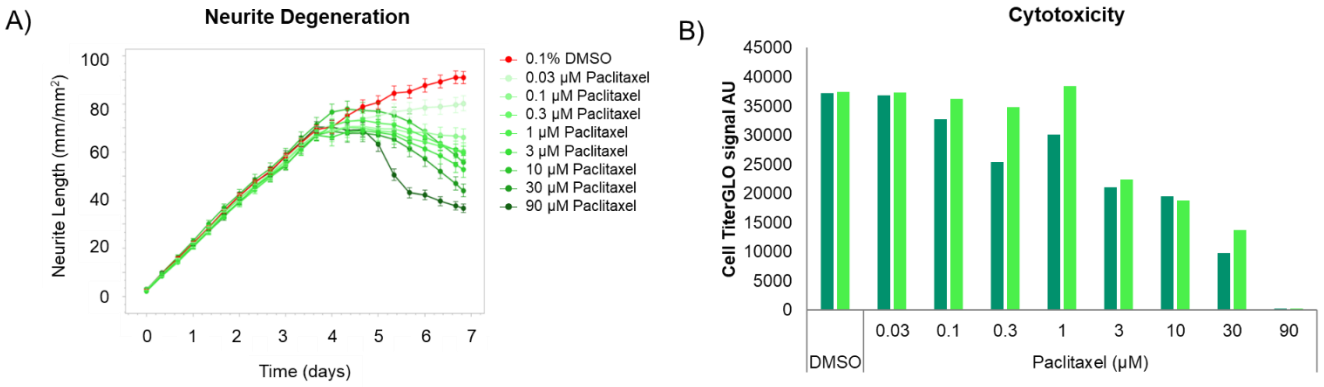


Figure 4: iCell Sensory Neurons Demonstrate Toxicity to CIPN Compounds

iCell Sensory Neurons, 01279 were plated following the protocol in the iCell Sensory Neurons User's Guide and cultured for 7 days. Treatment with Paclitaxel, a known chemotherapeutic with known chemotherapy-induced peripheral neuropathy (CIPN) side-effects, were applied at 4 days and neurite outgrowth and toxicity monitored out to day 7. ***A)*** *Increasing doses of Paclitaxel results in truncation of neurite outgrowth and induces neurite degeneration. The neurite outgrowth EC50 for Paclitaxel = 5 µM.* ***B)*** *Dose-dependent cell death in response to Paclitaxel was observed at day 7 (3 days of compound exposure) as measured by the Promega CellTiter-Glo®.*