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Value from Innovation

Cellular Dynamics

Modeling Glutamate-induced Excitotoxicity with iCell® GABANeurons

iCell Lab Note

Introduction.

Excitotoxicity is a process that occurs when high levels of molecules like glutamate, NMDA, or kainic acid agonists bind to glutamate receptors in post-synaptic neurons leading to cell injury or even death. This kind of neuronal toxicity is a complicating factor for many neurological disorders, including stroke, trauma, seizures, and epilepsy. Human iPSC-derived neurons have been established as a reliable source of biologically relevant cells for in vitro toxicity testing. This iCell® Lab Note provides a technical overview and representative data for using iCell GABANeurons to model glutamate-induced excitotoxicity with various cell health assay readouts.

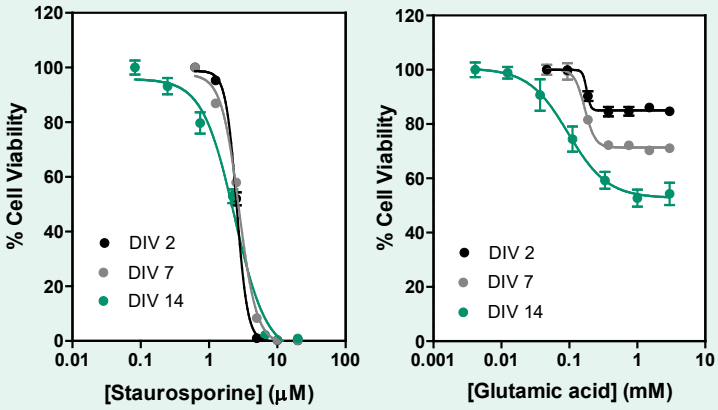

Figure 1. Comparing cytotoxicity to excitotoxicity. iCell GABANeurons were cultured until DIV 2, 7, or 14 and then dosed with either staurosporine or glutamic acid for 48 hours. Afterwards, cell viability was measured by CellTiter-Glo® assay. These data demonstrate that staurosporine is cytotoxic at any time point during the culture and that the cell viability is reduced to zero. However, since neurons require time in culture to establish synaptic networks, glutamate-induced excitotoxicity is not detectable until DIV 14 and the decrease in cell viability is about ~50% of control.

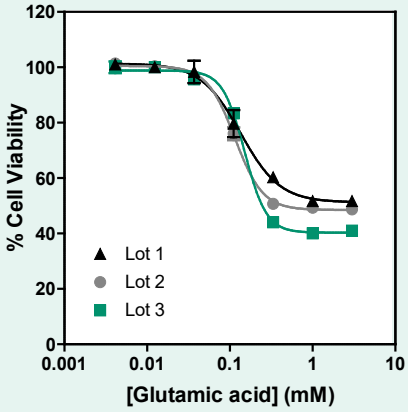

Figure 2. Lot-to-lost consistency. Three different lots of iCell GABANeurons were cultured in a PLO/ laminin-coated 96-well plate and tested on DIV 14 for glutamate-induced excitotoxicity (same conditions as in Figure 1). Dose-response curves highlight the reproducibility of the assay (with IC50 values of 0.135, 0.115, and 0.147 mM; normalized % cell viability at 1 mM glutamate = 51, 48, and 41). Slight variations in cell seeding density may account for the minor differences in the maximum amount of cell death observed.

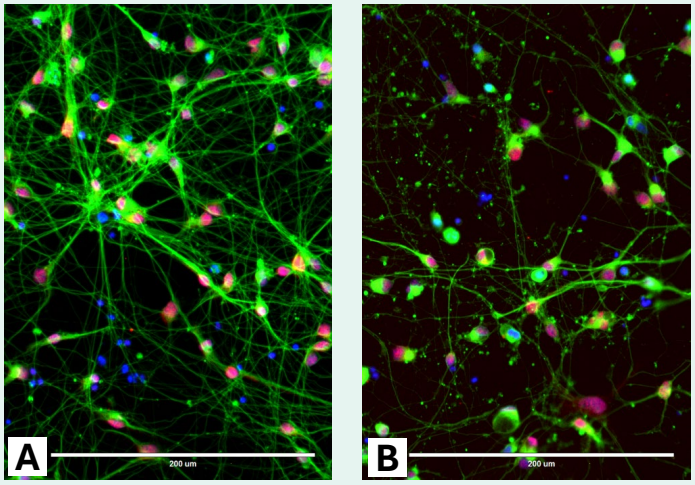

Figure 3. Cell morphology. Fluorescent images of iCell GABANeurons on DIV 14 either (**A**) untreated or (**B**) dosed with 1 mm glutamic acid for 48 hours. Cells were then fixed and stained with antibodies for TUJ1 (**green**), PSD95 (**red**), and nuclear dye Hoechst 33342 (**blue**). It is evident that cultures of synaptically interconnected human iPSC-derived neurons are disrupted and dying in the presence of the excitotoxic compound glutamic acid. Scale bar represents 200 µm.

Alternative Assay Readouts.

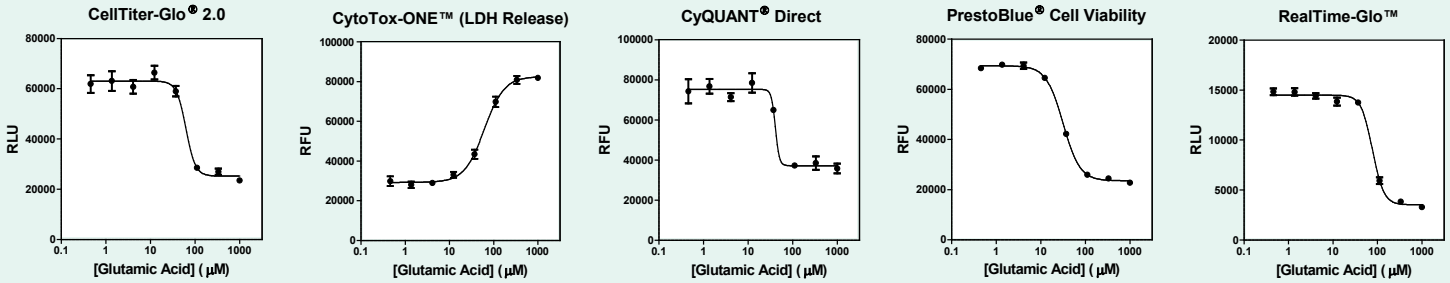

Figure 4. Multiple ways to measure cell viability. Representative DIV 14 assay data with iCell GABANeurons.

Methods.

Refer to the User's Guide for information on handling/storage of iCell GABANeurons and the medium/supplements.

- Prepare Complete Maintenance Medium by adding 2 ml of iCell Neural Supplement A (100X) to the 100 ml bottle of iCell Neural Base Medium.
- Coat a 96-well plate with PLO and then laminin (3.3 µg/ml diluted in PBS) according to the User's Guide.
- Thaw cells, spin to pellet, resuspend in complete medium, and seed 40K cells per well in 200 µl into 96-well plate.
- Perform 50% media change every 2-3 days until DIV 14.
- On the day of assay, prepare a 30 mM solution of Glutamic Acid (or glutamate) in complete medium.
- Prepare a serial dilution of glutamate and add to cells.
  - For example, adjust total volume of media in each well to 100 µl and transfer 50 µl of 3X compound to 96-well plate.
- Incubate cells with glutamate for 48 hours.
- Perform cell viability assay according to the manufacturer's instructions. Example endpoint assay readouts are listed in Table 1 and data is presented in Figure 4.
- Plot raw data or normalize 100% viability to untreated cells.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell GABANeurons, 01434 †	FCDI	C1012
▪ iCell Neural Base Medium	(incl. in kit)	M1010
▪ iCell Neural Supplement A	(incl. in kit)	M1054
Cell Culture Plate 96-well	Multiple	NA
Poly-L-Ornithine (PLO)	Sigma-Aldrich	P4957
Laminin	Sigma-Aldrich	L2020
Glutamic Acid	Sigma-Aldrich	G1501
CellTiter-Glo® 2.0 Cell Viability Assay	Promega	G9241
CytoTox-ONE™ Membrane Integrity Assay	Promega	G7890
CyQUANT® Direct Cell Proliferation Assay	ThermoFisher	C35011
PrestoBlue® Cell Viability Reagent	ThermoFisher	A13262
RealTime-Glo™ MT Cell Viability Assay	Promega	G9711

† Cells from donor 01434 were used to generate all data in this Lab Note. Similar performance from iCell GABANeurons, 01279 (C1008) is expected.

Summary.

This iCell Lab Note provides recommendations on how to measure excitotoxicity with iCell GABANeurons. Human iPSC-derived neurons are widely used to study this type of neurotoxicity due to their ability to recapitulate specific neuronal characteristics. During the development of these assays, time in culture to establish network connectivity was highlighted as critically important for assay success (see Figure 1). Glutamate is the primary excitatory neurotransmitter involved here, but other molecules like NMDA, AMPA, and kainate are also capable of overstimulation. Additionally, cell viability can be measured using various methods, each with its own advantages and applications.

References.

Chen, Y. (2018). Mechanistic Assessment of Excitotoxicity in Drug Discovery. InTech. doi: 10.5772/intechopen.77043.

Highlights.

Glutamate-induced excitotoxicity is a specific subtype of neurotoxicity that is culture duration dependent.

This protocol can be applied to other neuronal cell types in the FCDI portfolio, including iCell GlutaNeurons.

A series of different commercially available cell viability assay kits are compatible with this protocol.

Scan here to download the iCell GABANeurons User's Guide.

Contact **Technical Support** for more protocol details and supportive data.
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