



iCell® GlutaNeurons

Experimental models are essential scientific tools that continually evolve. iCell® GlutaNeurons from FUJIFILM Cellular Dynamics, Inc. (FCDI), are human glutamatergic-enriched neurons derived from induced pluripotent stem (iPS) cells. These cells provide a relevant excitatory neuronal model that overcomes the shortcomings of many other in vitro and ex vivo models.

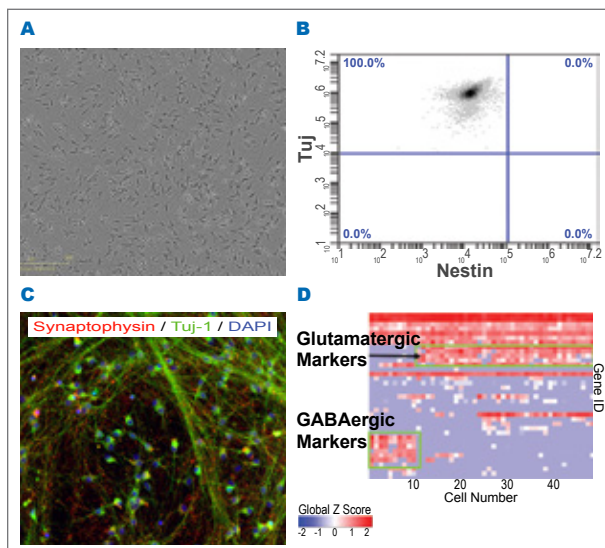
Specifically, iCell GlutaNeurons enable researchers to study human neuronal network development and activity through interrogation and manipulation of relevant pathological pathways involved in seizurogenic and neurodegenerative conditions, thereby providing a new and valuable tool for drug discovery, toxicity testing, and basic research.

Researchers have used various in vitro models in studying basic neuronal physiology and drug discovery.

Despite their use, traditional systems, such as primary cells from rodents, have significant drawbacks in terms of biological relevance, reproducibility, and scalability. FCDI has provided a solution to these problems by offering iCell GlutaNeurons. Available in consistent, commercial quantities, these primarily glutamatergic human cortical neurons display typical physiological characteristics and form functional neuronal networks amenable to examination in a number of standard assay techniques.

Advantages

- **Highly pure human cells:** Terminally differentiated from human iPS cells, iCell GlutaNeurons provide a uniquely relevant biological model.
- **Homogenous and reproducible:** Commercial quantities of consistent batches ensure reproducible large-scale screens and long-term projects.
- **Self-assembling networks:** The capability to form predominately excitatory neural networks provide a powerful tool in basic research and drug discovery studies.
- **Acute and long-term testing:** iCell GlutaNeurons remain viable and pure in culture for more than 4 weeks, enabling assessment of synapse formation as well as network development and disruption.
- **Easy to implement:** iCell GlutaNeurons are shipped cryopreserved with optimized media. Simply thaw and use.



▲ **Figure 1: iCell GlutaNeurons Provide a Highly Pure Population of Glutamatergic Cortical Neurons**
 (A) The cells display typical morphology, developing branched networks within 24 hours. (B) Flow cytometry data verify a highly pure, fully differentiated neuronal population. (C) Immunofluorescent labeling identifies the synaptic marker synaptophysin, neuronal marker tuj-1, and nuclei. (D) Single-cell gene expression analysis confirms the high proportion of glutamatergic neurons, which enables the formation of synchronously bursting networks.

Toxicity Characterization

iCell GlutaNeurons are amenable to a variety of standard assays and exhibit typical neuronal physiological

functions and responses that make them an ideal model for in vitro toxicity screening and drug development.

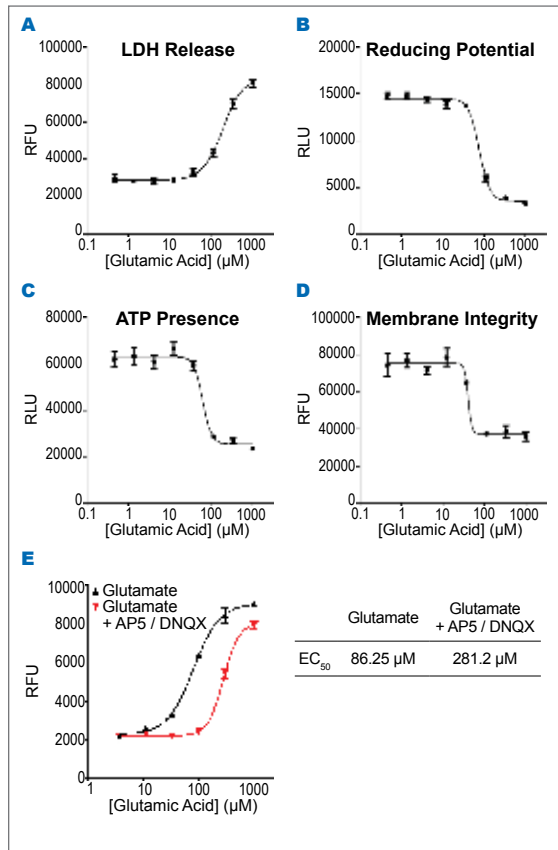


Figure 2: iCell GlutaNeurons Provide a Relevant Human-derived Model for Investigating Toxicity and Identifying Neuroprotectants

(A - D) Upon increased exposure to glutamate, iCell GlutaNeurons exhibit changes in their viability as assessed by, but not limited to, LDH release (CytoTox-ONE assay, Promega), reducing potential (RealTime-Glo assay, Promega), ATP presence (CellTiter-Glo assay, Promega), or membrane integrity (CyQUANT assay, Thermo Fisher Scientific), respectively. (E) Glutamate-induced cell death can be ameliorated by inhibition of NMDA (AP5) and AMPA (DNQX) receptors, as assessed by LDH release, highlighting the utility of iCell GlutaNeurons in screening for neuroprotectants.¹

¹ LDH = lactate dehydrogenase; ATP = adenosine triphosphate; NMDA = N-methyl-D-aspartate; AP5 = (2R)-amino-5-phosphonovaleric acid / (2R)-amino-5-phosphonopentanoate; AMPA = α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DNQX = 6,7-dinitroquinoxaline-2,3-dione

² PTZ = pentylenetetrazol; 4AP = 4-aminopyridine

Calcium Homeostasis

iCell GlutaNeurons evoke ligand-induced and spontaneous Ca^{2+} oscillations, providing a model system

for detecting disruptions in intracellular calcium levels that can lead to neuronal injury and death.

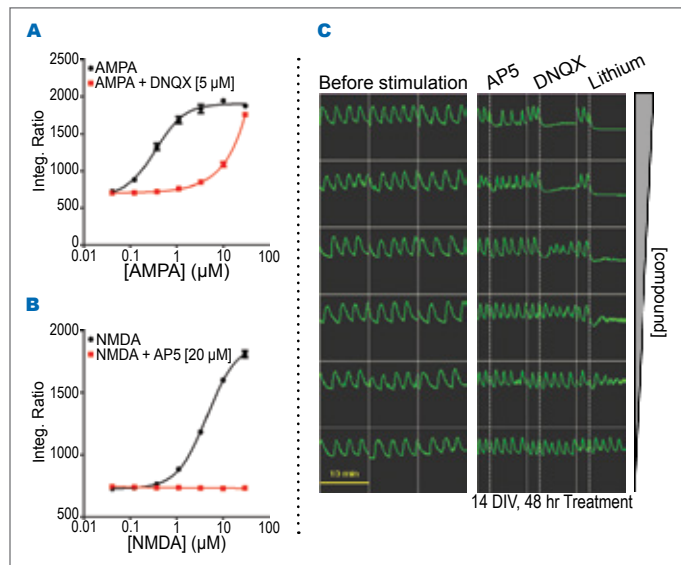


Figure 3: iCell GlutaNeurons Elicit Ligand-induced and Spontaneous Ca^{2+} Oscillations for Monitoring Compound Effects on Calcium Homeostasis
(A, B) Increasing the concentration of AMPA or NMDA induces Ca^{2+} influx that can be attenuated with the glutamate receptor inhibitors AP5 and DNQX, respectively. (C) Modulating spontaneous Ca^{2+} oscillations in iCell GlutaNeurons also enables high-throughput screening of relevant intracellular pathways.¹

Electrophysiological Characterization

iCell GlutaNeurons form spontaneously active excitatory neural networks that can be used to screen for

seizurogenic compounds and to understand and treat neurodegenerative conditions.

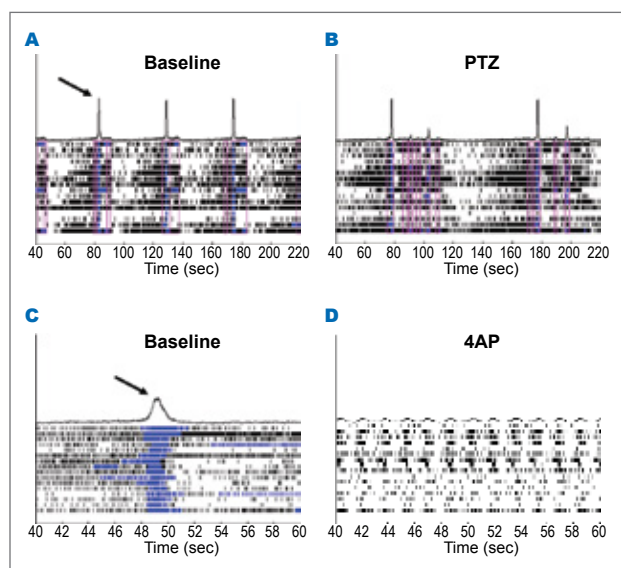


Figure 4: iCell GlutaNeurons Form Spontaneous Synchronous Networks for Modeling Epilepsy and Seizurogenic Toxicities
(A, C) Electrical activity recorded from a multielectrode array (MEA) illustrates the formation of highly connected networks (28 DIV). Analysis options include, but are not limited to, identifying synchronous bursting across the plate (pink boxes in the raster graph in panel A), burst intensity and duration highlighted by the waveform in the upper velocity graph (arrowheads), and action potential frequency above set thresholds (blue shading in the raster graphs). (B, D) The utility of iCell GlutaNeurons to serve as a model to study epilepsy and seizurogenesis is demonstrated by continued arrhythmic activity with GABA receptor block (PTZ) and increased low level bursting activity with K^+ channel block (4AP).²

Applications

iCell GlutaNeurons are amenable to a variety of uses including:

Cell-based Assays

- Cell viability
- Calcium signaling
- Neurite outgrowth and retraction

Electrophysiological Applications

- Identification and characterization of network function
- Higher throughput assessment of compound efficacy for seizure treatment
- Higher throughput detection of seizurogenic toxicity

Specifications

| | |
|------------------|--|
| Cell Type | Cortical neurons |
| Organism | Human |
| Source | Differentiated from an FCDI reprogrammed human iPS cell line |
| Quantity | $\geq 1.0 \times 10^6$ or $\geq 6.0 \times 10^6$ viable cells per vial |
| Shipped | Frozen |

Ordering Information

| Kit | Component(s) ^{1, 2} | Catalog Number |
|---------------------------------|--|----------------|
| iCell GlutaNeurons Kit, 01279 | $\geq 1.0 \times 10^6$ viable cells 2 ml Neural Supplement B 1 ml Nervous System Supplement | R1061 |
| | $3 \times \geq 1.0 \times 10^6$ viable cells 2 ml Neural Supplement B 1 ml Nervous System Supplement | R1116 |
| | $\geq 6.0 \times 10^6$ viable cells 2 ml Neural Supplement B 1 ml Nervous System Supplement | R1034 |
| iCell Neural Supplement B | 2 ml Neural Supplement B | M1029 |
| iCell Nervous System Supplement | 1 ml Nervous System Supplement | M1031 |

1 A User's Guide is provided in each kit.

2 These products were formerly known by these names and/or catalog numbers:

- iCell GlutaNeurons, 01279 = iCell GlutaNeurons (Cat. No. GNC-301-030-001)
- iCell Neural Supplement B = iCell DopaNeurons Medium Supplement (Cat. No. DNM-301-031-001)
- iCell Nervous System Supplement = iCell Nervous System Supplement (Cat. No. NSS-301-031-001)

For More Information

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iCell Products

Provide access to biologically relevant, human iPS cells for disease modeling, drug discovery, toxicity testing, and regenerative medicine. FCDI's rapidly growing portfolio of iCell products includes human cardiomyocytes, GABAergic, glutamatergic, dopaminergic and motor neurons, hepatocytes, endothelial cells, astrocytes, hematopoietic progenitor cells, skeletal myoblasts, macrophages, and others.

Visit the FCDI website for the most current list of supported cell types.

