**Introduction**

Human induced pluripotent stem (iPS) cells can proliferate infinitely and differentiate into most cell types in the human body. In addition, human iPS cells are a native cellular source similar to primary cell cultures that can be scaled to support large-scale screening applications. As human iPS cells are able to differentiate into many cell types, these features are attractive for assays to evaluate potential therapeutics and elucidating pathological conditions for therapeutic purposes.

ReproCELL has developed a comprehensive workflow that includes patient specific primary somatic cell isolation, cellular reprogramming, and genetic modification. To evaluate this workflow, we have used iPS cells for directed differentiation to the neural lineage. By regulating the differentiation conditions for these neurons, the proportion of neuronal subtypes can be controlled, and the resulting neurons can be analyzed functionally and phenotypically with MEA assays, ICC, and activity assays.

These derived neurons may also be created bearing Alzheimer’s or Parkinson’s disease-specific mutations, either by genetic modification, or by using iPS cells reprogrammed from disease patient cells. This comprehensive workflow capability enables us to generate customized disease models that target specific neurological disease requirements.

**Materials and Methods**

**ReproNeuro™ Kit** (ReproCELL, Cat. No. RCE5008)  
ReproNeuro MQ™ Kit (ReproCELL, Cat. No. RCE5010MQ)  
ReproNeuro DA plus™ Kit (ReproCELL)

**Immunocytochemistry**

**Equipment:** CellVoyager™ CV100 (Yokogawa Electric Corporation)

**Primary antibody**

- Anti-BIII-tubulin (Covance, Cat. Nos. MMS-435P, PRB-435P)
- Anti-Tyrosine hydroxylase (TH) (Abcam, Cat. No. ab5875)
- Anti-Choline acetyltransferase (ChAT) (Millipore, Cat. No. AB144)
- Anti-VGLUT1 (Sigma, Cat. No. V0389)
- Anti-GABA (Sigma, Cat. No. A2052)

**Secondary antibody**

- Alexa Fluor® 488 (Life Technologies, Cat. Nos. A11034, A10036)
- Alexa Fluor® 546 (Life Technologies, Cat. Nos. A11030, A11059)

**MEA Assay**

- Equipment: ME64-Basic (Alpha MED Scientific Inc.)

Reagent:

- D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5) (Abcam, Cat. No. ab120003)
- 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (WAKO, Cat. No. 03232121)
- Tetraiodo-5-[(2-phenylethyl)amino]-2′,4′-diaminodiphenyl ether-2-sulfonate (Tetrodotoxin (TTX)) (Abcam, Cat. No. ab120035)

**MPP+ Assay**

- T-methyl-4-phenylpyridinium iodide (MPP+), (Sigma-Aldrich, Cat. No. D048)
- Mag-Fura-2 (Life Technologies, Cat. No. M1720)
- Tetramethylrhodamine (TMRE) (Invitrogen, Cat. No. T-669)
- KMG-301 (ref., Shindo et al., Plas Dis 68b: 23664 (2011))

**Concept for Human iPS Cell-Derived Neurons**

- **Specialty services and cell-products of individual companies**
  - Human primary cell (Normal, Disease)
  - iPS cells without any genetic change
  - Differentiated cells customized tests
  - Disease models
  - Human tissue bank
  - Genetic engineering models

**Conclusion**

By regulating the differentiation conditions, iPS cell-derived neuron subpopulation composition and function can be controlled for different product characteristics.

Compared to the current ReproNeuro neurons:

- ReproNeuro DA plus neurons contain a higher proportion of dopaminergic neurons with a more robust MPP+ response.
- ReproNeuro MQ neurons have more spontaneous MEA activity and show higher-frequency spikes and greater sensitivity to antagonists.