Efficient Differentiation of Human Pluripotent Stem Cells into Neural Progenitor Cells

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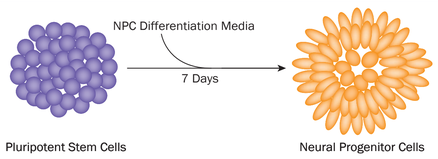
Abstract

Neurological disorders are the leading cause of human morbidity, accounting for over ten percent of all human disease. Traditional approaches for studying nervous system development, injury, and disease are largely limited to animal models, which often fail to fully recapitulate human disorders. Moreover, the variable etiology of a given neurological disease in humans demands a personalized approach to understanding and treating individual patients. Human pluripotent stem cells, including embryonic (ESC) and induced pluripotent stem cells (iPSCs), offer an essentially unlimited source of neural cells that can be used not only to study the mechanisms of human disease, but also as powerful tools for neural regeneration. Here, we introduce our StemXVivo® Neural Progenitor Differentiation Kit, which efficiently converts human pluripotent stem cells into neural progenitors. Within seven days, pluripotent stem cells are converted to Pax6-, SOX1-, and Nestin-positive neural progenitor cells. The derived neural progenitor cells (NPCs) can be expanded for several passages *in vitro* and differentiated into all three major cell types of the nervous system: neurons, astrocytes, and oligodendrocytes. These neural progenitors provide an intermediate multipotent stem cell population for further downstream differentiation, neuronal subtype derivation and mechanistic studies. The StemXVivo® Neural Progenitor Differentiation Kit provides a powerful platform for the reproducible generation of neural progenitor cells from diverse pluripotent human stem cell sources for use in disease modeling and drug discovery.

StemXVivo® Neural Progenitor Differentiation Kit

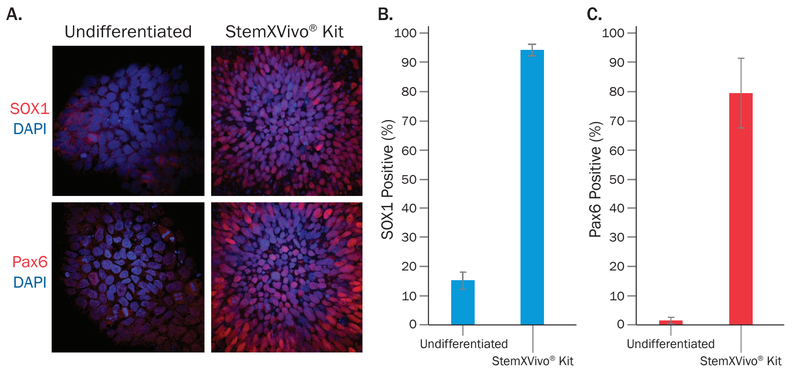
Kit Schematic



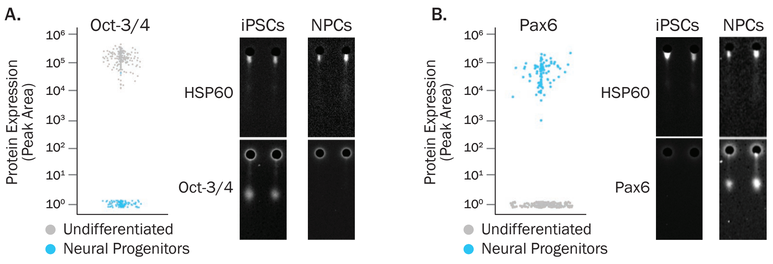
Kit Contents

- NPC Differentiation Base Media Supplement
- NPC Differentiation Cocktail
- Anti-SOX1 Antibody

Efficient Induction of Neural Progenitors

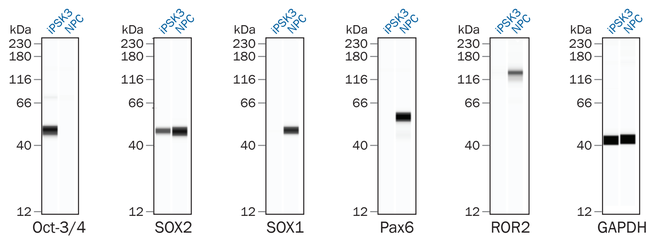


NPC Differentiation Efficiency Quantified Using High Content Imaging. The StemXVivo® Neural Progenitor Differentiation Kit was used to differentiate human iPSK3 (**A**) or JOY6 (**B**) iPSCs into NPCs. (**A**) Immunostaining of iPSK3 cells before (Undifferentiated) and after (StemXVivo® Kit) differentiation shows that kit-induced NPCs express characteristic neural progenitor markers, SOX1 (Catalog # AF3369) and Pax6 (Catalog # AF8150). (**B, C**) SOX1- and Pax6-positive cells were quantified in JOY6 cells both before (Undifferentiated) and after (StemXVivo® Kit) differentiation using high content imaging (Operetta, Perkin-Elmer). Kit-differentiated cells were over 90% positive for SOX1 and over 75% positive for Pax6. In contrast, undifferentiated cells were less than 20% positive for SOX1 and 5% positive for Pax6.

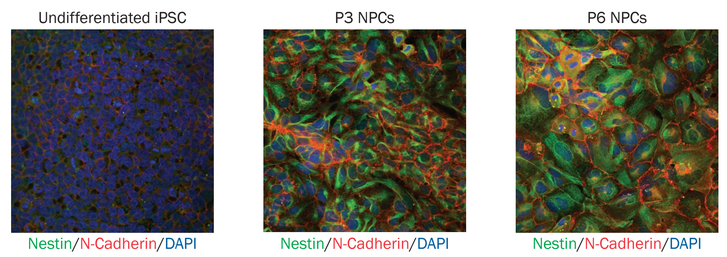


Kit-derived NPCs Homogenously Express Pax6. JOY6 iPSCs (Undifferentiated) and StemXVivo® Neural Progenitor Differentiation Kit-derived NPCs (Neural Progenitors) were probed at the single-cell level for Oct-3/4 (**A**) and Pax6 (**B**) expression using Milo™ Single-Cell Western technology (ProteinSimple). Scatter plots demonstrate that undifferentiated iPSCs homogenously express Oct-3/4 (% positive cells), while kit-derived NPCs homogenously express Pax6 and lack Oct-3/4 expression. Each dot in the scatter plot represents protein expression level within one cell. Representative single-cell Western Blot images are shown for Oct-3/4 and Pax6 in undifferentiated iPSCs and NPCs. HSP60 was included as a loading control.

Pluripotent Stem Cell-derived NPCs Express Characteristic NPC Markers

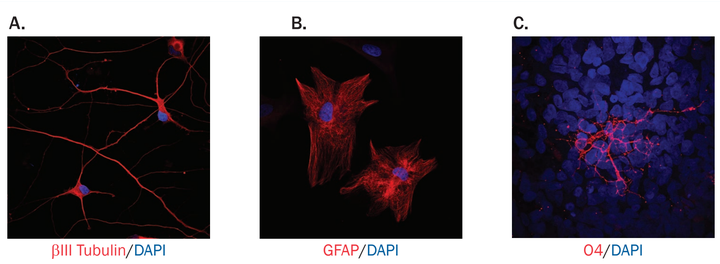


Kit-derived NPCs Express SOX1, Pax6, and ROR2. Cell lysates from iPSK3 cells either before (iPSK3) or after (NPC) differentiation using the StemXVivo® Kit were analyzed by Simple Western™ for expression of pluripotent- and NPC-specific markers. Undifferentiated iPSK3 cells express high levels of Oct-3/4 (Catalog # AF1759) and SOX2 (Catalog # AF2018), while kit-differentiated NPCs have upregulated expression of SOX1, Pax6, and ROR2 (Catalog # AF2064). GAPDH (Catalog # MAB5718) was used as a loading control.



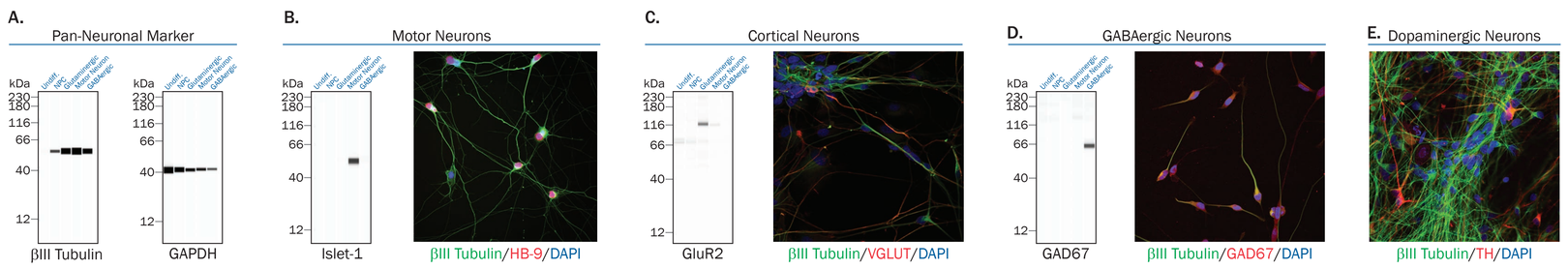
Kit-derived NPCs Maintain Neural Progenitor Cell Markers Over Multiple Passages. Kit-derived NPCs were maintained in culture for up to 6 passages. At passage (P)3 and P6, NPCs were analyzed for expression of Nestin (Catalog # MAB1259) and N-Cadherin (Catalog # AF6426). Cell nuclei were stained with DAPI (Tocris, Catalog #5748). Undifferentiated iPSCs were used as a control and do not express Nestin.

Kit-derived NPCs Differentiate into Neurons, Astrocytes, and Oligodendrocytes



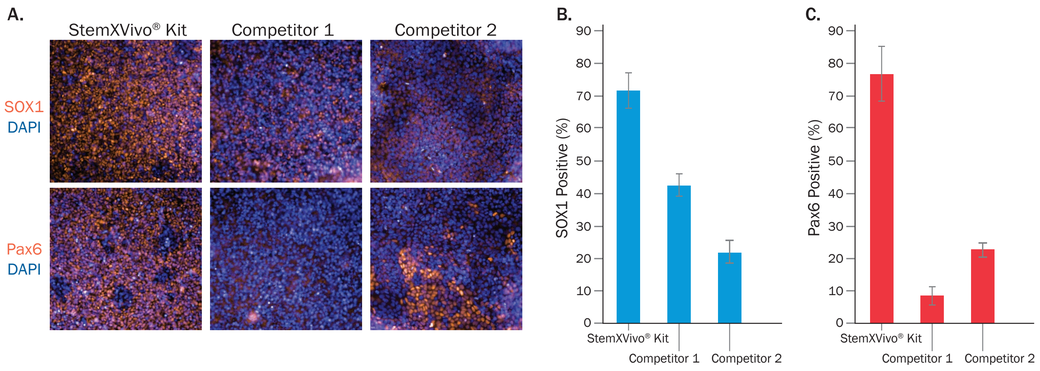
Pluripotent Stem Cell-derived NPCs are Multipotent. JOY6 human iPSCs were differentiated into NPCs using this kit. NPCs were further differentiated into neurons (**A**), astrocytes (**B**), and oligodendrocytes (**C**) via growth factor withdrawal. Cells were stained with the Mouse Anti-Neuron-Specific βIII Tubulin Monoclonal Antibody (Neurons; Catalog # MAB1195), Sheep Anti-Human GFAP Polyclonal Antibody (Astrocytes; Catalog # AF2594), and Mouse Anti-Human Oligodendrocyte Marker O4 Monoclonal Antibody (Oligodendrocytes; Catalog # MAB1326) followed by NorthernLights™ (NL)557-Conjugated Donkey Anti-Mouse, Donkey Anti-Sheep, or Goat Anti-Mouse Secondary Antibodies, respectively (Catalog # NL007, NL010, or NL019). Cell nuclei were stained with DAPI.

Differentiation of NPCs into a Diverse Range of Neuronal Lineages



NPCs Derived from JOY6 iPSCs using the StemXVivo® Kit were Further Differentiated into Downstream Neuronal Lineages using Standard Protocols. (**A**) Simple Western™ analysis confirms that differentiation into Glutamatergic, GABAergic, and Motor Neurons generates βIII Tubulin-expressing neurons. GAPDH was used as a loading control for all Simple Western™ data shown. (**B**) Motor neuron differentiation was confirmed by Simple Western™ analysis for Islet-1 (Catalog # AF1837) and immunocytochemistry for HB-9 (Novus Biologicals, Catalog # NBP2-24691). (**C**) Cortical glutamatergic neuron differentiation was confirmed by Simple Western™ analysis for GluR2 and immunocytochemistry for VGLUT (Catalog # MAB9054). (**D**) GABAergic neuron differentiation was confirmed for GAD67 (Catalog # AF2086) expression using Simple Western™ analysis and immunocytochemistry for cells double positive for GAD67 and βIII Tubulin (Catalog # NL1195). (**E**) Dopaminergic neuron differentiation was confirmed via immunocytochemistry for Tyrosine Hydroxylase (TH, Catalog # MAB7566), and βIII Tubulin.

StemXVivo® Kit is More Robust than Current Commercially Available NPC Differentiation Kits



StemXVivo® Neural Progenitor Differentiation Kit Has Superior Performance Compared to Other Commercially Available Kits. JOY6 human iPSCs were differentiated into NPCs using either the StemXVivo® Kit or other commercially available kits (Competitor 1 & Competitor 2). Differentiation efficiency was determined by staining for SOX1 and Pax6 expression. (**A**) Representative images of SOX1 and Pax6 staining from NPCs derived using the StemXVivo® Kit, Competitor 1, or Competitor 2. (**B, C**) SOX1 and Pax6 expression levels were quantified using high-content analysis. The data demonstrate that the StemXVivo® Kit results in a higher percentage of SOX1- and Pax6-positive NPCs in comparison to the other commercially available kits.

Conclusions

- The StemXVivo® Neural Progenitor Differentiation Kit efficiently differentiates human pluripotent cells into neural progenitor cells.
- Single-Cell Western blotting confirms NPC population homogeneity following differentiation.
- The NPCs derived using this kit maintain the ability to differentiate into the three neural lineages; neurons, astrocytes, and oligodendrocytes.
- Kit-derived NPCs can be further differentiated into specific neuronal subtypes including Motor Neurons, Glutamatergic neurons, GABAergic neurons, and Dopaminergic Neurons.
- The StemXVivo® Neural Progenitor Differentiation Kit out-performs other commercially available kits.