# Reproducible and Efficient Differentiation of Human Pluripotent Stem Cells to Pancreatic Progenitors Using a Novel Serum-Free Medium

M.J. Riedel[1], S. Lam[1], Y. Luu[1], T.E. Thomas[1], A.C. Eaves[1,2], and S.A. Louis[1]
[1] STEMCELL Technologies Inc., Vancouver, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, B.C., Canada

## Introduction

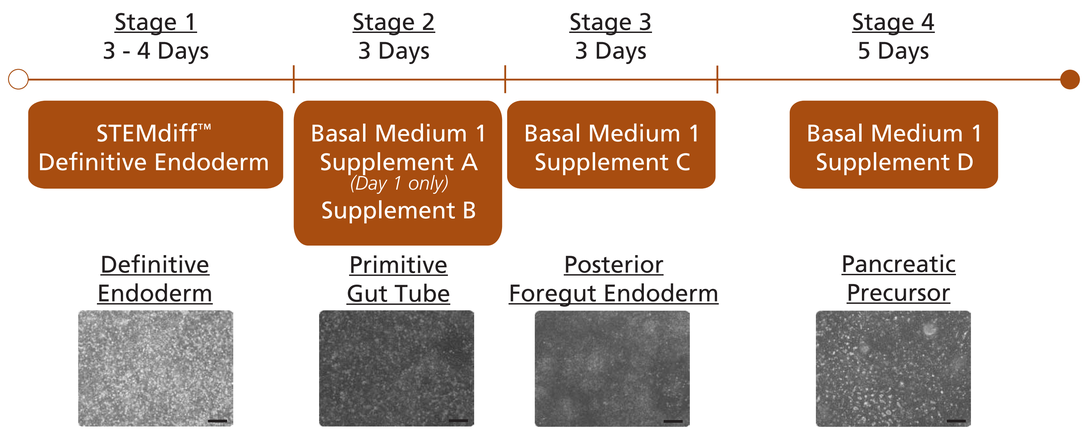
Type 1 diabetes is characterized by the loss of the insulin-producing beta cells of the pancreatic islets. Infusing cadaveric donor islets into the portal circulation of type 1 diabetic individuals can induce insulin independence. However, a shortage of donor islets precludes this cell therapy from widespread clinical use and typically only those individuals who cannot control their glucose homeostasis by exogenous insulin delivery qualify for this procedure.

An alternative source of tissue for transplantation may be insulin-producing cells derived from human pluripotent stem cells (hPSCs). Recent advances in protocols for the derivation of pancreatic cell types from hPSCs have resulted in the initiation of clinical trials in the United States, whereby immature pancreatic progenitor cells are transplanted within a device implanted under the skin (Kroon *et al.*, Nature Biotechnology, 2008 and Schulz *et al.*, PLOS ONE, 2012). Maturation of these precursor cells to functional endocrine cells occurs over the course of months in mouse models and it is anticipated that a similar maturation of these cells will occur in humans. Additional research programs are now aimed at developing protocols to mature these pancreatic precursor cells *in vitro* as well as using standardized protocols for generating pancreatic cells for disease modeling and developmental studies.

Several protocols exist to generate pancreatic precursors from hPSCs, but with varying efficiency and reproducibility across hPSC lines. To standardize generation of hPSC-derived pancreatic precursors, we have developed the STEMdiff™ Pancreatic Precursor Differentiation Kit, consisting of a defined medium and supplements that supports efficient and reproducible generation of pancreatic precursors from multiple hPSC lines. Cells differentiated using this optimized medium and protocol expressed key markers including PDX-1, NKX6.1, PTF1α, NEUROD1, and NGN3, and will provide researchers with a standardized tool for pancreatic cell studies.

## Protocol

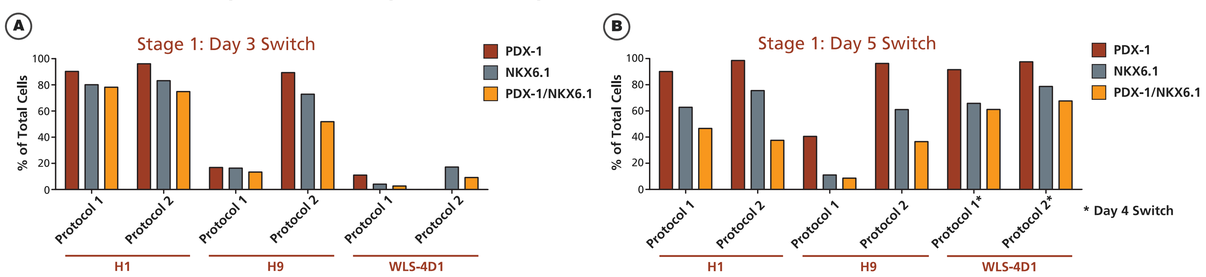
**FIGURE 1: Overview of the differentiation protocol and the changes in cell morphology over time**




The STEMdiff™ Pancreatic Precursor Differentiation Kit is used downstream of the STEMdiff™ Definitive Endoderm Kit. In the 4 stages of differentiation to pancreatic cells, hPSCs will transition through definitive endoderm (Stage 1), primitive gut tube (Stage 2) and posterior foregut endoderm (Stage 3) as they become pancreatic precursors (end Stage 4). Stage 1 differentiation utilizes the STEMdiff™ Definitive Endoderm Kit. Transition timing to Stage 2 is cell line-dependent. Most cell lines can be transitioned after day 3; however, some human induced pluripotent stem cell (hiPSC) lines may require one additional day in Stage 1 (**Figure 2**). The remainder of the protocol utilizes a single basal medium and a series of 4 supplements to promote the formation of pancreatic precursor cells in a multistep protocol. Representative images of cell morphology are shown at the end of each stage of differentiation. Scale bar = 50 µm.

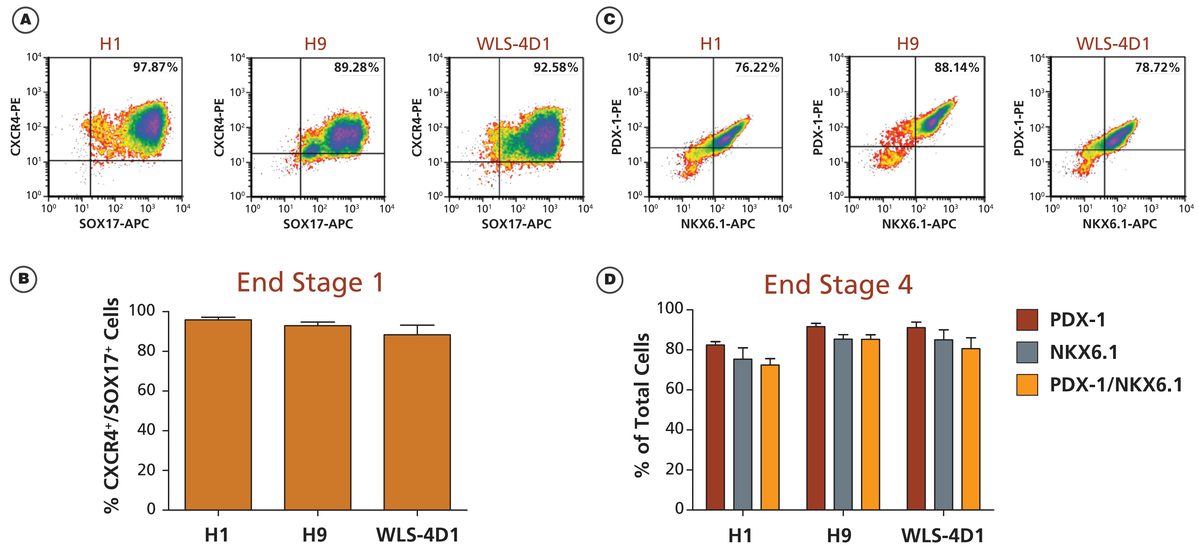
## Results

**FIGURE 2: Published protocols are optimized for specific cell lines**



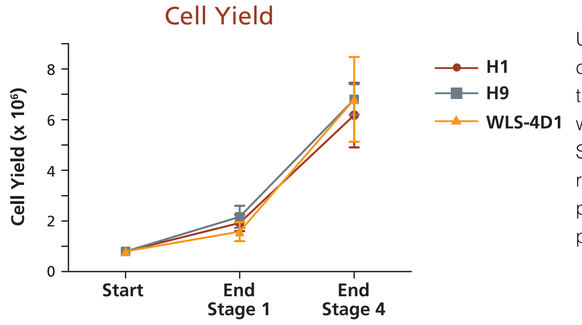

Using hPSCs maintained in mTeSR™1 on Matrigel® and differentiated to definitive endoderm using the STEMdiff™ Definitive Endoderm Kit, we investigated the optimal timing of transitioning the differentiating cells from Stage 1 to Stage 2. Protocols 1 and 2 are based on adaptations of two published protocols. Differentiation efficiency was assessed by quantifying the number of PDX-1$^+$/NKX6.1$^+$ cells at the end of Stage 4 by flow cytometry. In general, human embryonic stem cell (hESC) lines performed better when transitioned earlier (Day 3). The hiPSC line WLS-4D1 performed poorly when transitioned on Day 3, but improved significantly when transitioned on Day 4. Delaying transition of most cell lines to Day 4 (not shown) or Day 5 significantly reduced efficiency of differentiation, particularly by reducing NKX6.1 expression. Neither published protocol, when used in conjunction with our protocol modifications, yielded highly efficient differentiation across the three hPSC lines tested.

**FIGURE 3: STEMdiff™ Pancreatic Precursor Differentiation Kit functions efficiently across multiple hPSC lines**



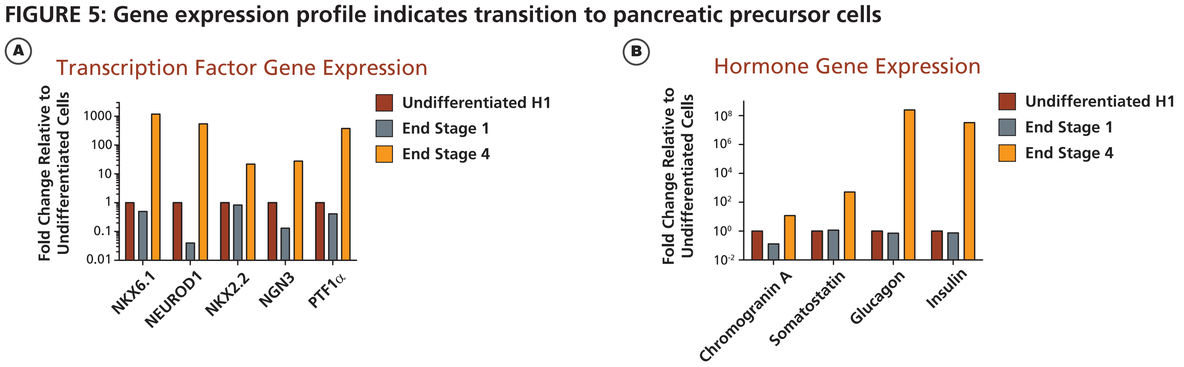

**A, B)** The STEMdiff™ Definitive Endoderm Kit generates CXCR4$^+$/SOX17$^+$ definitive endoderm with high efficiency across multiple cell lines. **A)** Representative flow cytometry plots for CXCR4 and SOX17 expression. **B)** Cumulative quantitative data for CXCR4 and SOX17 co-expression (mean ± SD; n = 2 - 3 per cell line in duplicate). **C, D)** Cells were immediately carried forward from the end of Stage 1 into Stages 2 - 4 without passaging, resulting in a highly efficient conversion of definitive endoderm cells into PDX-1$^+$/NKX6.1$^+$ pancreatic precursors. **C)** Representative flow cytometry plots for PDX-1 and NKX6.1 expression at the end of Stage 4. **D)** Cumulative quantitative data for PDX-1 and NKX6.1 co-expression (mean ± SD; n = 2 - 3 per cell line in duplicate). The efficiency of differentiation ranged from 72.5% to 85.3% depending on the cell line. The efficiency of conversion from definitive endoderm to pancreatic precursor ranged from 77.3% to 96.3%. In addition, nearly all NKX6.1$^+$ cells also expressed PDX-1 as observed in the developing human pancreas (Riedel *et al.*, Diabetologia, 2012).

**FIGURE 4: Rapid expansion yields high numbers of pancreatic precursors**




Undifferentiated hPSCs were seeded at 800,000 cells (2.1 x 10$^5$ cells/cm$^2$) into wells of a 12-well culture plate. By the end of Stage 1, there was an approximate 2-fold increase in total cell number, of which most were CXCR4$^+$/SOX17$^+$ definitive endoderm. By the end of Stage 4, the number of pancreatic precursors (PDX-1$^+$/NKX6.1$^+$ cells) ranged from 3.6 x 10$^6$ to 9.1 x 10$^6$ cells, yielding 4.5 to 11.4 pancreatic precursor cells per input hPSC. Data plotted as mean ± SD; n = 2 - 3 per cell line in duplicate.

**FIGURE 5: Gene expression profile indicates transition to pancreatic precursor cells**




Gene expression profile of **A)** key transcription factors or **B)** hormones expressed in pancreatic precursor cells. Expression was first normalized to TATA Binding Protein (TBP) and then to the expression level found in undifferentiated cells. Gene expression is shown at the end of Stage 1 and at the end of Stage 4. Data are the mean of duplicate technical replicates for a single experiment on H1 cells. Expression pattern is consistent with published data (Rezania *et al.*, Nature Biotechnology, 2014).

## Summary

- STEMdiff™ Pancreatic Precursor Differentiation Kit promotes efficient and reproducible generation of NKX6.1$^+$/PDX-1$^+$ pancreatic progenitor cells.
- Compatible with the STEMdiff™ Definitive Endoderm Kit, this novel medium allows for efficient pancreatic precursor generation from multiple human pluripotent stem cell lines.
- Pancreatic precursors generated with the STEMdiff™ Pancreatic Precursor Differentiation Kit exhibit a gene expression profile similar to state-of-the-art published protocols, including up-regulation of key transcription factors NKX6.1, NEUROD1 and NGN3.
- The STEMdiff™ Pancreatic Precursor Differentiation Kit consists of a serum-free medium and supplements in a simple format with an easy-to-follow protocol.