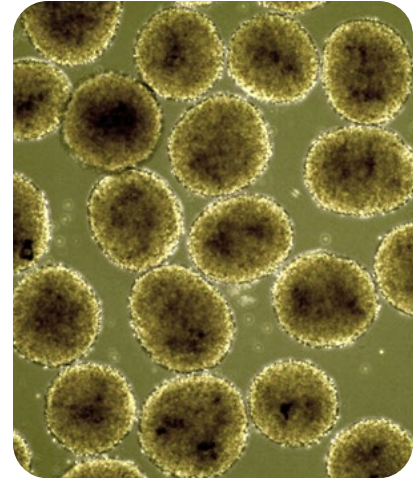


Generation and Characterization of iPSC-derived Insulin-Producing *Pancreatic Beta Islets*

Introduction

Diabetes is a chronic condition resulting from the loss or dysfunction of insulin-producing pancreatic beta cells, which leads to an inability to properly regulate blood glucose levels. Diabetes affects over 500 million people worldwide and is commonly managed through insulin injections or oral drugs, but stem cell-based therapy may offer a promising new approach for treatment. This method uses pluripotent stem cells to generate insulin-producing pancreatic beta cells, which mature to form islets that can be transplanted into a diabetic patient to replace the dead or damaged cells and restore their function. If successful, this approach would provide a renewable, consistent supply of insulin-producing beta cells and have the potential to remove the dependence on routine insulin injections for diabetic patients trying to manage their disease.

To help accelerate the development of a stem cell-based therapy for diabetes, we show that R&D Systems™ reagents and instruments can be used to support beta cell manufacturing workflows from cell culture to characterization. Using a differentiation protocol adapted from Hoglebe, et al. (2021), human induced pluripotent stem cells (iPSCs) were differentiated with GMP proteins and small molecules into functional pancreatic beta islets. A high differentiation efficiency was achieved and found to be reproducible over multiple experiments, as indicated by the expression of target cell markers at defined stages throughout the differentiation protocol. In the final cell population, 75-80% of the cells stained double positive for the C-peptide and NKX6.1 beta cell markers, confirming a high degree of beta cell enrichment. Further characterization using the Simple Plex™ Ella Automated ELISA platform showed that the beta islets secrete insulin following glucose stimulation at levels comparable to those reported in the literature for functional beta islets.¹ Together, these results demonstrate that R&D Systems reagents can be used to efficiently differentiate and characterize iPSC-derived pancreatic beta cells, which are critical steps in the development of a stem cell-based therapy for diabetes.



Key Takeaways

- R&D Systems reagents and instruments can be used to support successful beta cell manufacturing workflows from cell culture to phenotyping and functional characterization.
- Differentiation to pancreatic beta cells was highly efficient and reproducible over multiple experiments.
- The final cell population was highly enriched for beta cells, with 75-80% of the cells consistently staining double positive for the markers C-peptide and NKX6.1.
- The beta islets secreted insulin following glucose stimulation, at levels comparable to those reported in the literature for functional beta islets.

Materials

R&D Systems Products for iPSC Maintenance and Expansion

Product Name	Catalog No.
ExCellerate™ iPSC Expansion Medium, Animal-Free, GMP	CCM036-GMP
Cultrex™ UltiMatrix Reduced Growth Factor Basement Membrane Extract	BME001-05
Recombinant Human FGF basic Heat Stable GMP Protein	BT-FGFBHS-GMP
Y-27632 dihydrochloride, GMP	TB1254-GMP

R&D Systems Products for Pancreatic Beta Cell Differentiation

Product Name	Catalog No.
ExCellerate™ iPSC Expansion Medium, Animal-Free, GMP	CCM036-GMP
Y-27632 dihydrochloride, GMP	TB1254-GMP
CHIR 99021, GMP	TB4423-GMP
Recombinant Human/Mouse/Rat Activin A GMP Protein	338-GMP
Recombinant Human KGF/FGF-7 GMP Protein	BT-KGF-GMP
Retinoic acid	TB0695-RMU
LDN 193189, GMP	TB6053-GMP
TPPB	5343
SANT-1	1974
DAPT	TB2634-RMU
RepSox	TB3742-RMU
T3	TB6666-RMU
Compound E	6476
Lantrunculin A	3973
L-Ascorbic acid	TB4055-RMU
Heparin sodium salt	2812

R&D Systems or Novus Biologicals Products for Cell Lineage Characterization

Product Name	Catalog No.
APC-conjugated Rat Anti-Human/Mouse Oct-3/4 Monoclonal Antibody	IC1759A
PE-conjugated Goat Anti-Human Nanog Antigen Affinity-purified Polyclonal Antibody	IC1997P
FlowX FoxP3/Transcription Factor Fixation & Perm Buffer Kit	FC012
APC-conjugated Goat Anti-Human SOX17 Antigen Affinity-purified Polyclonal Antibody	IC1924A
Alexa Fluor® 488-conjugated Goat Anti-Human HNF-3 beta/FoxA2 Antigen Affinity-purified Polyclonal Antibody	IC2400G
PE-conjugated Mouse Anti-Human/Mouse PDX-1/IPF1 Monoclonal Antibody	IC2419P
PE-conjugated Mouse Anti-Human Chromogranin A Monoclonal Antibody	NBP2-34795PE
DA ZP1	7444
Mouse Anti-Human C-Peptide Monoclonal Antibody	MAB14171
Rat Anti-Human/Mouse Somatostatin Monoclonal Antibody	MAB2358
NorthernLights™ NL493-conjugated Donkey Anti-Mouse IgG Antigen Affinity-purified Polyclonal Antibody	NL009
NorthernLights™ NL557-conjugated Goat Anti-Rat IgG Antigen Affinity-purified Polyclonal Antibody	NL013

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R&D Systems Products for Functional Characterization of Beta Islets

Product Name	Catalog No.
Simple Plex™ Human Insulin Cartridge	SPCKB-PS-000507
Ella Automated Immunoassay System	600-100

Experimental Workflow

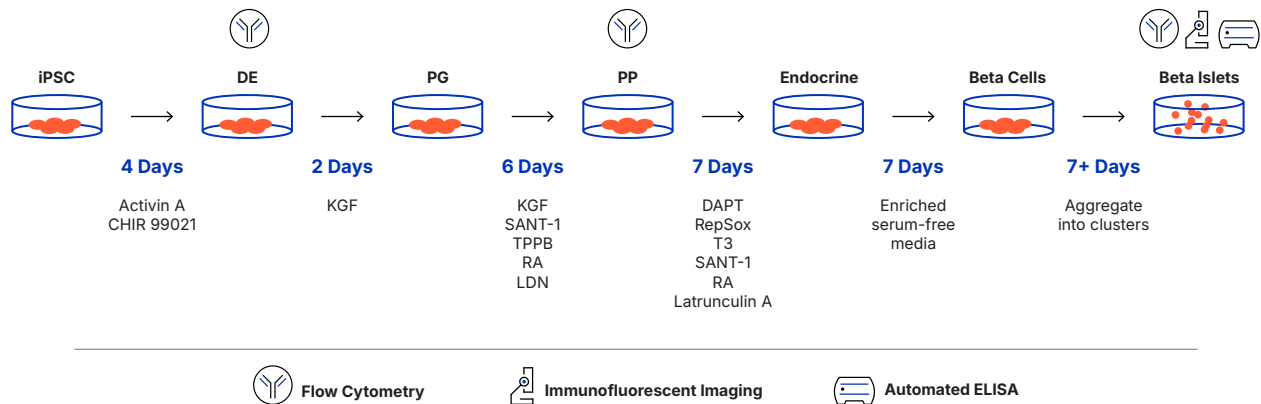
A protocol adapted from Högberg, *et al.* (2021) was used to generate functional human pancreatic beta islets from induced pluripotent stem cells (iPSCs).¹ Briefly, iPSCs were seeded in a 24-well plate and allowed to reach 95-100% confluency before the differentiation was initiated (Figure 1). Differentiation media was exchanged every 24-48 hours depending on the stage of differentiation. To produce beta islets, the beta cells were detached from the culture plate and resuspended in maintenance media, followed by 3D culture on an orbital shaker for further maturation.

Throughout the protocol, brightfield microscopy and flow cytometry were used to characterize the cell morphologies and phenotypes at defined stages of development, including definitive endoderm (DE), primitive gut tube (PG), pancreatic progenitors (PP), endocrine cells, and beta cells. Flow cytometry, brightfield microscopy, immunofluorescent imaging, and a Simple Plex Human Insulin Automated ELISA were used to characterize the final beta islets.

The yield using this 35-day differentiation protocol was approximately $2 - 3 \times 10^7$ beta cells for each 24-well plate of iPSCs. The beta islets could be further expanded beyond Day 35 depending on the downstream applications.

FIGURE 1

Differentiation Workflow for Human iPSC-derived Pancreatic Beta Islets



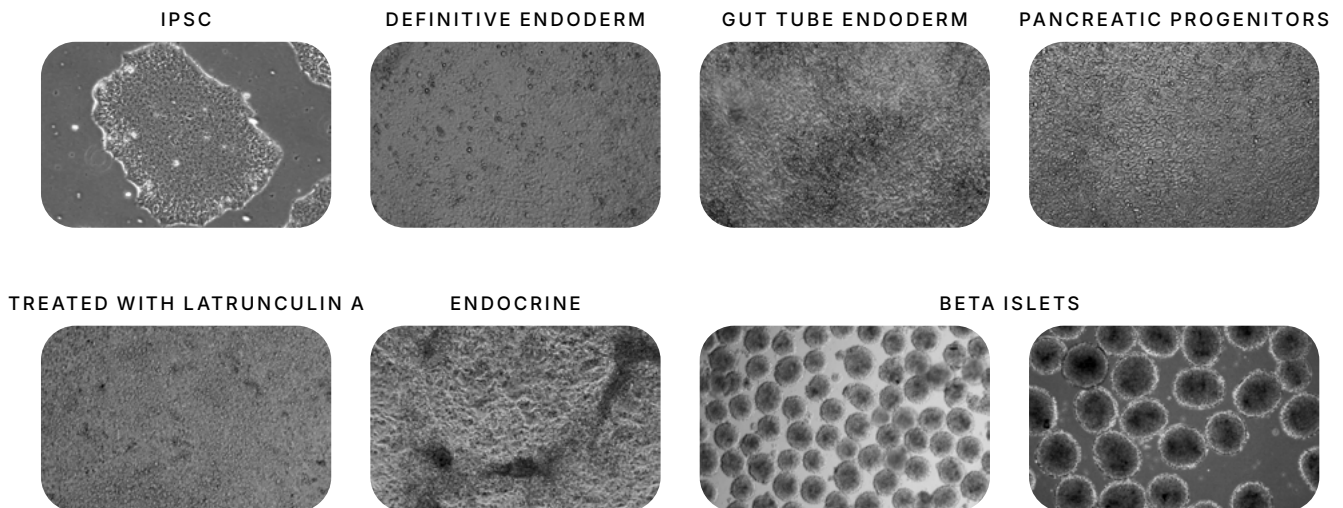
The workflow used in this study was adapted from a protocol described in Högberg *et al.*, where a human induced pluripotent stem cell (iPSC) line was differentiated into insulin-secreting pancreatic beta islets. The 35-day differentiation encompasses 5 major intermediate stages: 1) definitive endoderm (DE); 2) primitive gut tube (PG); 3) pancreatic progenitors (PP); 4) pancreatic endocrine; and 5) beta cells. The source and catalog numbers for each of the reagents included in the differentiation workflow are listed in the Materials section. Throughout the workflow, the defined stages of development were characterized using a variety of techniques, including brightfield microscopy, flow cytometry, immunofluorescent imaging, and functional ELISA.

Results

Brightfield microscopy and flow cytometry were used to characterize the key intermediary stages of cell differentiation throughout the 35-day protocol used to generate pancreatic beta islets from iPSCs. As shown in the data, the cells displayed the expected morphology (Figure 2) and expressed high levels of the expected markers (Figure 3) at each of the key differentiation steps. A high differentiation efficiency was achieved as $\geq 90\%$ of the cells in the definitive endoderm cell population were SOX17⁺/FOXA2⁺; $\geq 80\%$ of the cells in the pancreatic progenitor cell population were PDX-1⁺/NKX6.1⁺; $\geq 80\%$ of the cells in the pancreatic endocrine cell population were Chromogranin A⁺; and 75-80% of the cells in the final cell population stained double positive for the beta cell markers, C-peptide and NKX6.1. Significantly, the high differentiation efficiency and target cell yield could be consistently reproduced over multiple differentiation experiments, demonstrating that the culture conditions could be easily replicated from one experiment to the next with R&D Systems reagents.

FIGURE 2

Cell Morphologies at Each Stage of Differentiation



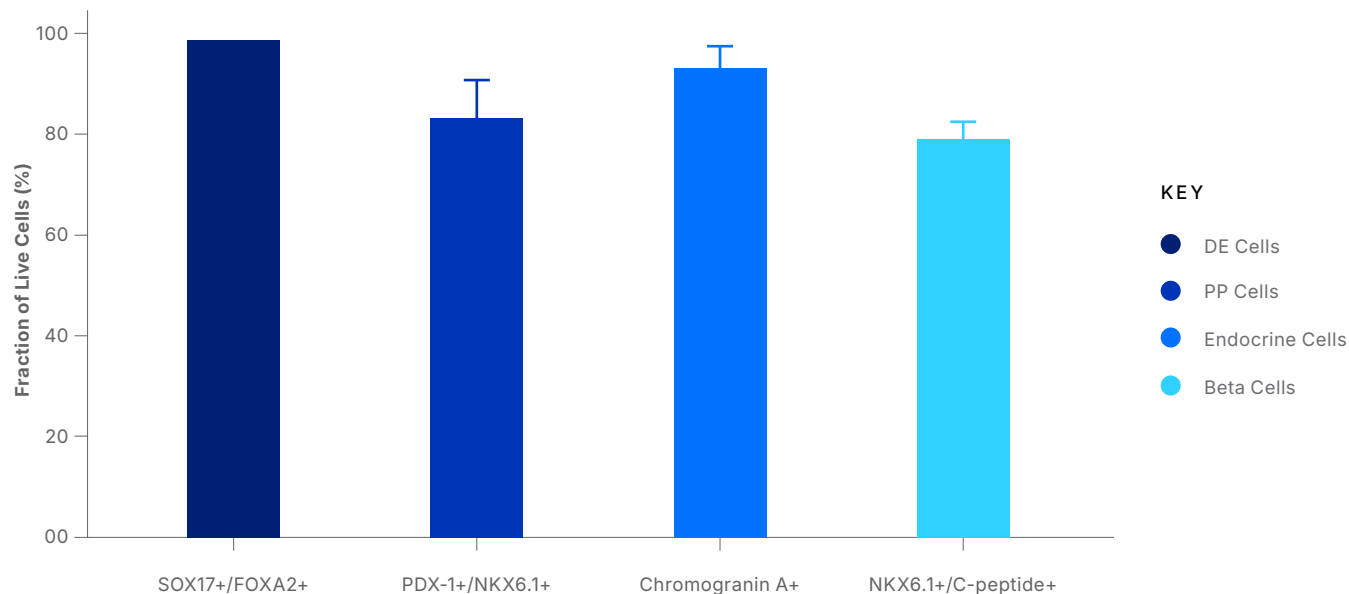
Representative brightfield images of cells taken during the 35-day differentiation workflow. Cells grew as monolayers through the endocrine stage. Then, beta cells were aggregated into clusters and expanded in suspension to produce beta islets. Notably, the mature iPSC-derived beta islets showed a homogeneous aggregate size.

To confirm the successful differentiation of pancreatic beta islets at the end of the 35-day differentiation protocol, immunofluorescent staining was performed using antibodies against C-peptide, a marker of insulin-secreting beta cells and somatostatin, a marker of delta cells, in the pancreatic islets (Figure 4). The resulting data showed that C-peptide was expressed in most of the cells, while somatostatin expression was observed in a scattered subpopulation of delta cells and their nearby tissues. The heterogeneity of the cell populations was typical for mature and functional insulin-secreting pancreatic islets. Notably, the structure of the cell aggregates was whole and did not show a necrotic core, indicating the high quality of the cells.

To functionally characterize the pancreatic beta islets generated with this protocol, their ability to produce insulin was assessed using a static glucose-stimulated insulin secretion assay. Following incubation with 20 mM glucose for one hour, insulin levels in the cell culture supernatants were analyzed using R&D Systems Simple Plex Human Insulin Automated ELISA on the Ella™ platform (Figure 5). This analysis clearly demonstrated that the beta islets could secrete insulin following glucose stimulation and the insulin levels were comparable to those reported in the literature for functional beta islets.¹

FIGURE 3

Characterization of Cellular Markers at Key Stages of Differentiation using Flow Cytometry

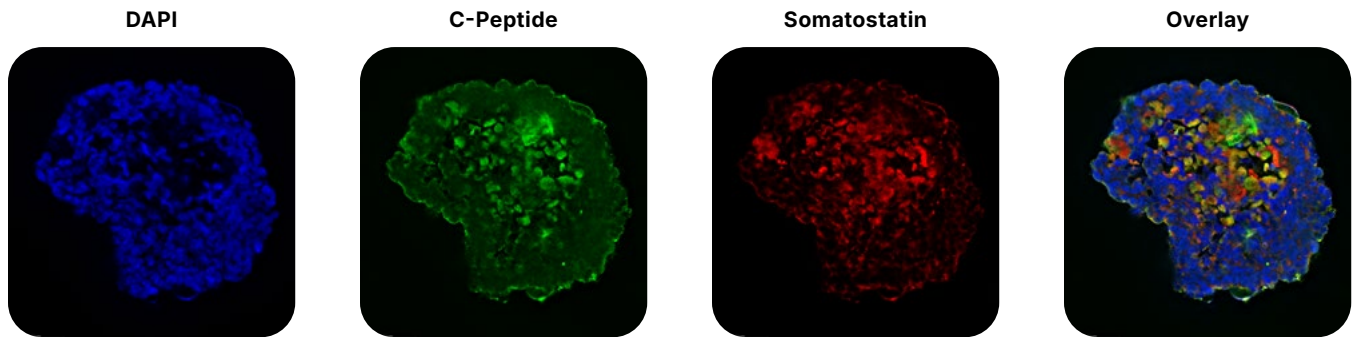


Following each key differentiation step, cells were fixed, permeabilized, stained for markers of the expected cell phenotype, and analyzed by flow cytometry. Definitive endoderm (DE) cells were stained using an **APC-conjugated Goat Anti-Human SOX17 Antigen Affinity-purified Polyclonal Antibody** (R&D Systems, Catalog # IC1924A) and an **Alexa Fluor® 488-conjugated Goat Anti-Human FOXA2 Antigen Affinity-purified Polyclonal Antibody** (R&D Systems, Catalog # IC2400G). Pancreatic progenitor (PP) cells were stained using a **PE-conjugated Mouse Anti-Human/Mouse PDX-1 Monoclonal Antibody** (R&D Systems, Catalog # IC2419P) and an Alexa Fluor 647-conjugated mouse anti-human NKX6.1 monoclonal antibody. Endocrine cells were stained using a **PE-conjugated Mouse Anti-Human Chromogranin A Monoclonal Antibody** (Novus Biologicals, Catalog # NBP2-34795PE), and beta cells were stained using fluorescent-conjugated mouse anti-human C-peptide and NKX6.1 monoclonal antibodies. The differentiation to pancreatic beta cells was highly efficient as indicated by the high expression of the expected cellular markers at each differentiation stage.

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FIGURE 4

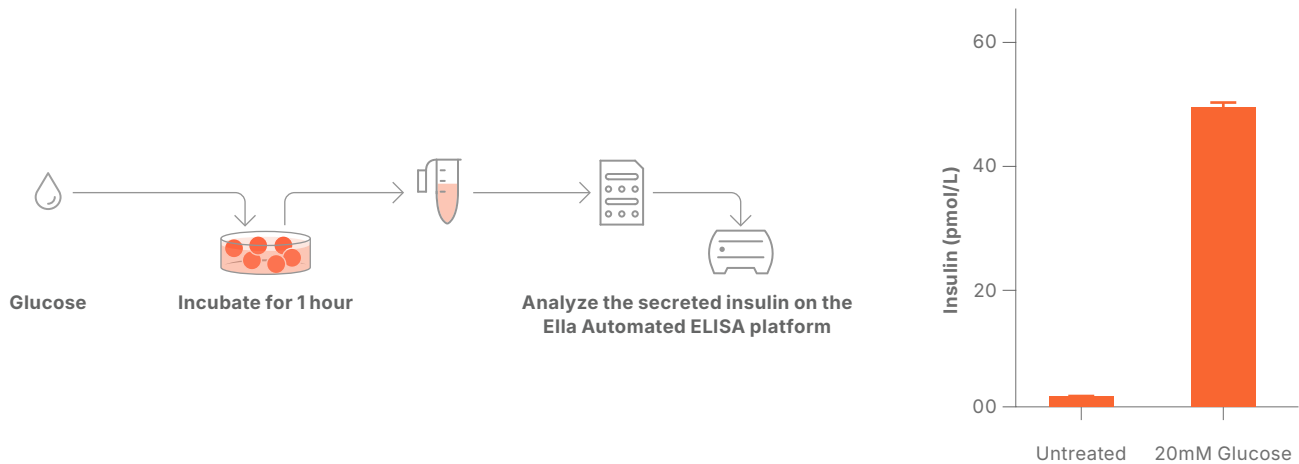
Immunofluorescent Staining of the iPSC-derived Beta Islets



Representative images of cells stained for characteristic markers of beta islets. iPSC-derived beta islets were fixed, frozen, and sectioned. Sections were stained with **DAPI** (Catalog # 5748), a **Mouse Anti-Human C-peptide Monoclonal Antibody** (R&D Systems, Catalog # MAB14171), and a **Rat Anti-Human/Mouse Somatostatin Monoclonal Antibody** (R&D Systems, Catalog # MAB2358), followed by secondary antibody staining with the **NorthernLights NL493-conjugated Donkey Anti-Mouse IgG Antigen Affinity-purified Polyclonal Antibody** (R&D Systems, Catalog # NL009) and **NorthernLights NL557-conjugated Goat Anti-Rat IgG Antigen Affinity-purified Polyclonal Antibody** (R&D Systems, Catalog # NL013). Expression of the beta cell marker, C-peptide in the majority of the cells and the delta cell marker, somatostatin in a scattered subpopulation of the cells confirmed the successful differentiation of pancreatic beta islets at the end of the 35-day differentiation protocol.

FIGURE 5

Functional Characterization of the iPSC-derived Beta Islets



Beta islets were collected after 35 days of culture for the static glucose-stimulated insulin secretion assay. Beta islets were plated in a 96-well plate and incubated with 20 mM glucose for 1 hour at 37 °C. After incubation, the cell culture supernatant was collected and analyzed using the **Simple Plex Human Insulin Cartridge** (R&D Systems, Catalog # SPCKB-PS-000507) on the **Ella™ Automated Immunoassay System** (R&D Systems, Catalog # 600-100). The data is represented as the average of three technical replicates +/- SD. The results showed that the beta islets could secrete insulin following glucose stimulation at levels comparable to those reported in the literature for functional beta islets.

Conclusion

In this study, we demonstrate the use of R&D Systems reagents and instruments to differentiate and characterize iPSC-derived, insulin-producing pancreatic beta islets. The differentiation efficiency achieved was high, with 75-80% of the cells in the final cell population staining double positive for the beta cell markers, C-peptide and NKX6.1. Functional characterization of the beta islets showed that they could secrete insulin following glucose stimulation, and importantly, the results were reproducible over multiple experiments. Therefore, we conclude that R&D Systems reagents can: 1) effectively and consistently generate pancreatic beta cells from iPSCs, 2) phenotypically characterize the cells at defined stages of differentiation, and 3) assess the functionality of the final beta islet population, which are three key steps in the pancreatic beta cell manufacturing process being pursued to develop a stem cell-based therapy for the treatment of diabetes.

REFERENCES

1. Högberg, N.J. *et al.* (2021) *Nat. Protoc.* **16**:4109.



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