A fully defined animal component-free medium for efficient differentiation of human pluripotent stem cells to definitive endoderm

Michael J. Riedel1, Stephanie Lam1, Terry E. Thomas1, Allen C. Eaves2, and Sharon A. Louis1

1 STEMCCELL Technologies Inc., Vancouver, BC, Canada 2 Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Introduction

The formation of definitive endoderm (DE) from human pluripotent stem cells (hPSCs) is a required intermediate step in the development of more specialized cell types of endoderm organs including the pancreas and liver. Current state of the art protocols for DE differentiation typically require the use of fetal bovine serum, an undefined component that may present a barrier to clinical translation. Furthermore, variability in the efficiency of DE differentiation among different hPSC lines may be the result of including such undefined components. The significance of this variability may be increased in the therapy of induced pluripotent stem cells (iPSCs), where each line may respond quite differently to the protocol. Given the increasingly widespread interest in developing disease models through the use of iPSCs, it is critical that a standardized protocol be developed that reduces variability across different hPSC lines.

To address the need for a standardized protocol for the differentiation of hPSCs to DE, we sought to develop an animal component-free (ACF) medium formulation that demonstrates efficient differentiation in multiple human embryonic (hES) and hiPSC cell lines. Here we introduce STEMdiff™ Definitive Endoderm, a fully defined, ACF kit for the directed differentiation of hPSCs to definitive endoderm. We show that this kit yields efficient differentiation in multiple hES and hiPSC cell lines, expressing several markers of DE including CXCR4, SOX17, and FOXA2. Furthermore, we demonstrate the ability of the DE formed using STEMdiff™ Definitive Endoderm to be further differentiated towards both pancreatic and hepatic lineages using published protocols. Finally, we show that efficient differentiation of hPSCs to DE can be achieved using cells maintained in the animal protein-free TeSR™2 medium and differentiated on recombinant human vitronectin, thus creating a fully defined system for definitive endoderm differentiation.

Product Format and Protocol

STEMdiff™ Definitive Endoderm Kit consists of a 100 ml bottle of basal medium and two 100X supplements

Day 0

Basal medium with STEMdiff™ Definitive Endoderm Condition Medium A (1X without supplement included in kit)

Day 1

Wash cells once with STEMdiff™ Definitive Endoderm Condition Medium B (1X without supplement included in kit)

Day 2

Remove medium and add STEMdiff™ Definitive Endoderm Condition Medium B (1X without supplement included in kit)

Day 3

Remove medium and add STEMdiff™ Definitive Endoderm Condition Medium B (same procedure as Day 2)

Day 4

Array for definitive endoderm markers by flow cytometry or immunostaining.

Results

Representative density plots showing CXCR4 and SOX17 expression in hES (H9) and hiPSC (AD1) cells following 4 days of differentiation to definitive endoderm using STEMdiff™ Definitive Endoderm. Isotype controls were used to set quadrant gates.

FIGURE 1: Efficient definitive endoderm differentiation in both hES and hiPSC cells

Representative images of CXCR4 and FOXA2 immunostaining in hES (H9) and hiPSC (AD1) cells following 4 days of differentiation to definitive endoderm using STEMdiff™ Definitive Endoderm. Merged images show extensive co-localization of these two markers. Scale bar, 100 μm.

FIGURE 2: Expression of key definitive endoderm markers in hES and hiPSC cells is widespread

Quantitative analysis of SOX17 and FOXA2 immunostaining in hES (H9) and hiPSC (AD1) cells following 4 days of differentiation to definitive endoderm using STEMdiff™ Definitive Endoderm. Merged images show extensive co-localization of these two markers. Scale bar, 100 μm.

FIGURE 3: Definitive endoderm differentiation is efficient across multiple hES and hiPSC cell lines

Quantitative analysis of SOX17 expression following 7 days in STEMdiff™ Definitive Endoderm. Merged images show extensive co-localization of these two markers. Scale bar, 100 μm.

FIGURE 4: Marker expression follows transition from pluripotency to definitive endoderm

Quantitative analysis of marker expression at daily intervals during differentiation of hPSCs to definitive endoderm. OCT4, SSEA-3, SOX17, and FOXA2 expression levels were monitored daily. OCT4 expression declined rapidly, while FOXA2 expression increased, indicating a transition from pluripotency to definitiveness.

Summary

- STEMdiff™ Definitive Endoderm is a fully defined, animal component-free medium formulation that allows for reproducible differentiation of hPSCs to definitive endoderm.
- Differentiation is efficient and reproducible across multiple hES and hiPSC cell lines, yielding cells that express multiple markers of definitive endoderm including CXCR4, SOX17, and FOXA2. These cells maintain their ability to be further directed towards pancreatic and hepatic lineages.
- STEMdiff™ Definitive Endoderm is compatible with cells maintained in either animal protein-free TeSR™2 or BSA-containing mTeSR™1 maintenance media.
- Differentiation is equally efficient on human recombinant vitronectin or Matrigel.
- STEMdiff™ Definitive Endoderm can reliably be used as a starting point for studies aimed at the formation of endoderm cell lineages from iPSCs.