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

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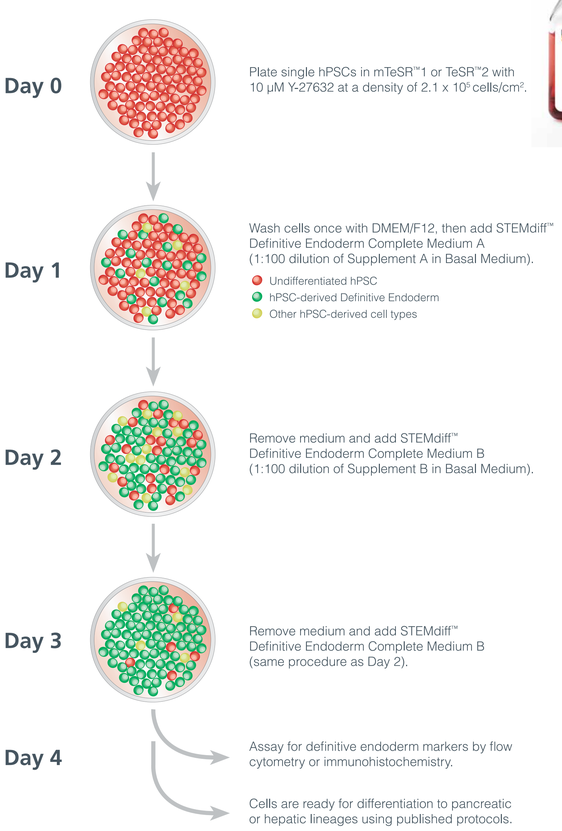
## Introduction

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 theatre of induced pluripotent stem (hiPS) cells, where each line may respond quite differently to the protocol. Given the increasingly widespread interest in developing disease models through the use of hiPS cells, 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 hiPS cell lines. Here we introduce STEMdiff™ Definitive Endoderm, a fully defined, ACF kit for the directed differentiation of hPSCs to multipotent DE. We show that this kit yields efficient differentiation in multiple hES and hiPS cell lines, expressing several markers of DE including CXCR4, SOX17, and FOXA2. Furthermore, we demonstrate the ability of the DE formed using STEMdiff™ Defintiive 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.

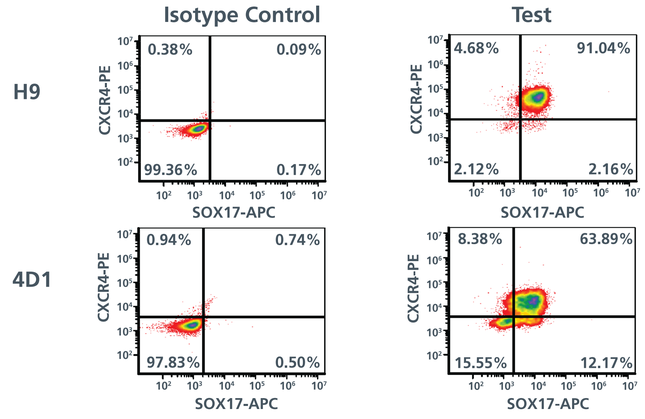
## Product Format and Protocol

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

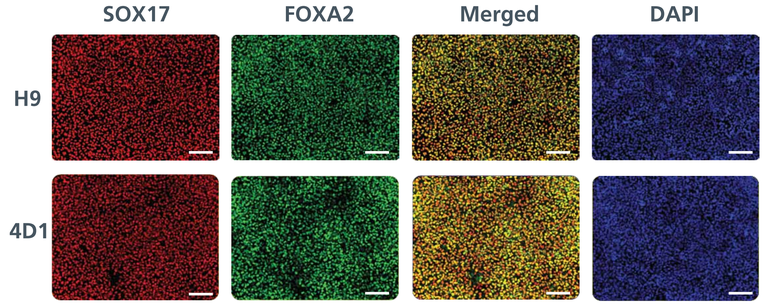

## Results

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



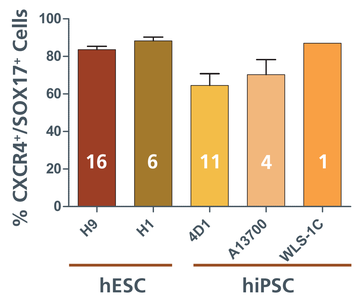

Representative density plots showing CXCR4 and SOX17 expression in hES (H9) and hiPS (4D1) cells following 4 days of differentiation to definitive endoderm using STEMdiff™ Definitive Endoderm. Isotype controls were used to set quadrant gates.

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



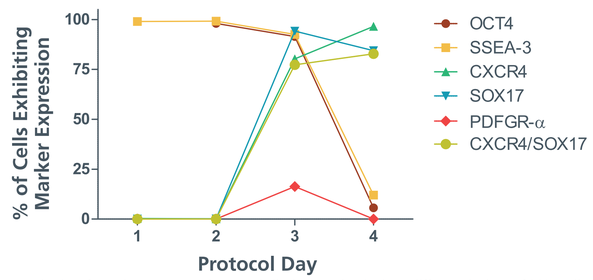

Representative images of SOX17 and FOXA2 immunoreactivity in hES (H9) and hiPS (4D1) cells following 4 days of differentiation to definitive endoderm using STEMdiff™ Definitive Endoderm. Merged images show extensive co-localization of these two markers. Scale bar, 100 µm.

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



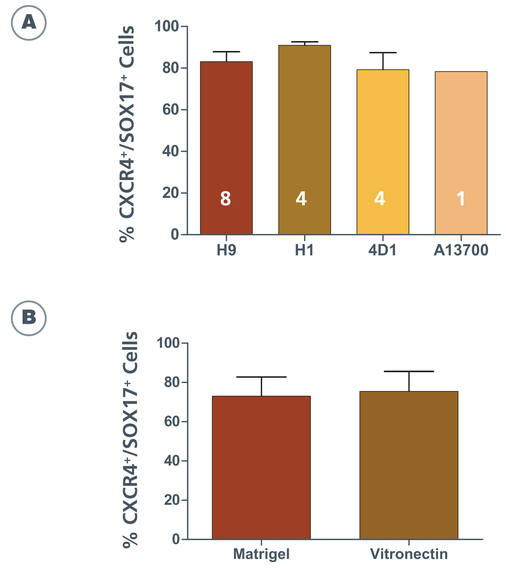

Quantitative analysis of definitive endoderm formation in multiple hPSC lines as measured by co-expression of CXCR4 and SOX17. Prior to differentiation using STEMdiff™ Definitive Endoderm, cells were maintained in their pluripotent state by culturing in mTeSR™1 on Matrigel. Data are expressed as the mean percent of cells expressing both markers. Error bars indicate SEM; n values for each cell line are indicated by the white number within each bar.

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



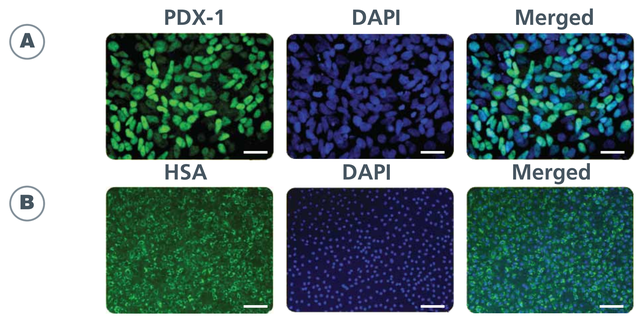

Quantitative analysis of marker expression at daily intervals during differentiation of H9 cells to definitive endoderm. Cells were harvested on each day of differentiation and examined for expression of pluripotency (OCT4, SSEA-3), definitive endoderm (CXCR4, SOX17), or mesoderm (PDFGR-α) markers. CXCR4 and SOX17 expression are robustly increased on Day 3, with co-expression of these two markers peaking on Day 4. Pluripotency markers remain elevated on Day 3 but are dramatically reduced on day 4, as is the mesoderm marker PDGFR-α.

### FIGURE 5: STEMdiff™ Definitive Endoderm is compatible with defined maintenance media and matrices




Ⓐ Quantitative analysis of Day 4 definitive endoderm in hPSCs previously maintained in TeSR™2 prior to differentiation on Matrigel using STEMdiff™ Definitive Endoderm. Data are expressed as the mean percent of cells expressing both markers. Error bars indicate SEM; n values for each cell line are indicated by the white number within each bar. Ⓑ Quantitative analysis of H9 cells differentiated using STEMdiff™ Definitive Endoderm on Matrigel or recombinant human vitronectin. Data are expressed as the mean percent of cells expressing both markers. Error bars indicate SEM; n=2 for each condition.

### FIGURE 6: STEMdiff™ Definitive Endoderm yields DE that retains potency for downstream lineage specification




Cultures differentiated using STEMdiff™ Definitive Endoderm maintain their ability to be directed towards pancreatic and hepatic lineages. Ⓐ Representative image of PDX-1 immunoreactivity in H9 cells following pancreatic specification. Scale bar, 20 µm. Ⓑ Representative image of human albumin (HSA) immunoreactivity in H9 cells following hepatic specification. Scale bar, 100 µm.

## 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 hiPS 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 hPSCs.