Highly efficient differentiation of human pluripotent stem cells to multipotent definitive endoderm using the serum-free and animal component-free STEMdiff™ Definitive Endoderm Culture System

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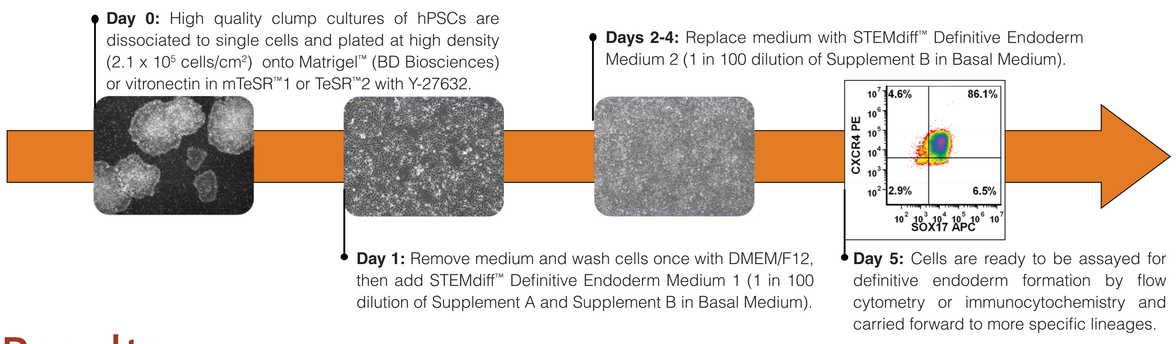
Introduction

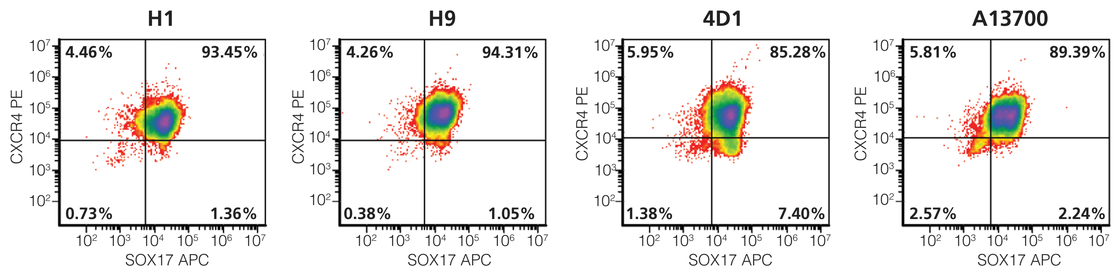
The formation of definitive endoderm (DE) from human embryonic stem (hES) and human induced pluripotent stem (hiPS) cells is a required intermediate step in the development of more specialized cells of endoderm organs. Recent studies show that hES and hiPS cells can be differentiated towards cell types of the liver, pancreas, lung, and intestine. Critical in these processes is the initial development of a highly pure population of DE. Current state-of-the-art protocols for DE differentiation often require fetal bovine serum to allow for high efficiency differentiation across multiple hPSC lines. However, inclusion of animal-derived serum may contribute to inconsistent performance and create a barrier to clinical translation. Furthermore, the use of patient-specific hiPS cells for disease modeling, drug screening, and cell-based therapies will require a protocol for efficient DE differentiation across multiple hiPS cell lines. To meet the need in the field for improved and standardized reagents and protocols for DE differentiation, we developed STEMdiff™ Definitive Endoderm, a fully defined serum-free and animal component-free medium that promotes highly efficient DE differentiation across multiple hPSC lines. Cells derived using STEMdiff™ Definitive Endoderm express high levels of DE-specific markers including CXCR4, SOX17, and FOXA2 and show reduced expression of pluripotent markers OCT4 and SSEA-3. Reduced expression of SOX2 following differentiation to DE suggests a lack of biasing towards anterior foregut endoderm lineages. Subsequent directed differentiation towards both anterior and posterior foregut endoderm using published protocols indicates that cells derived using STEMdiff™ Definitive Endoderm retain multipotency and can therefore be reliably used in studies aimed at generating cells of pulmonary, hepatic, or pancreatic lineages.

Product Format and Protocol



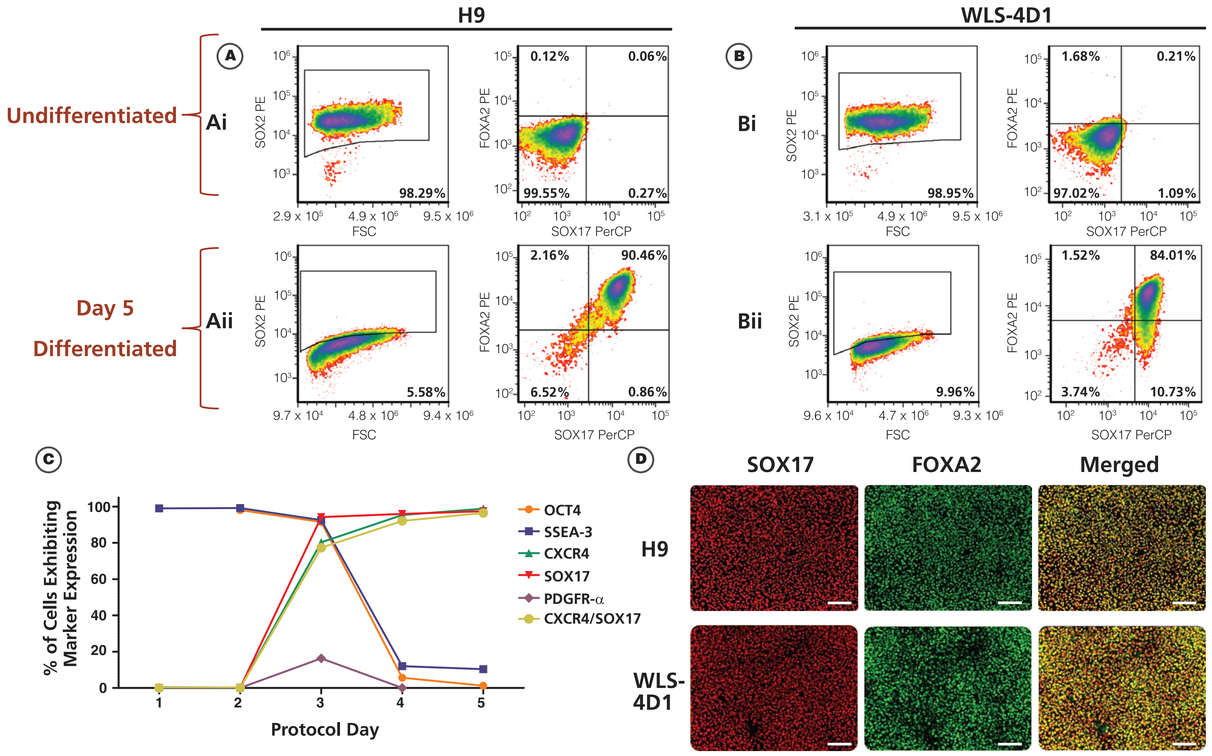
Results

FIGURE 1: Efficient differentiation to definitive endoderm in multiple hES and hiPS cell lines



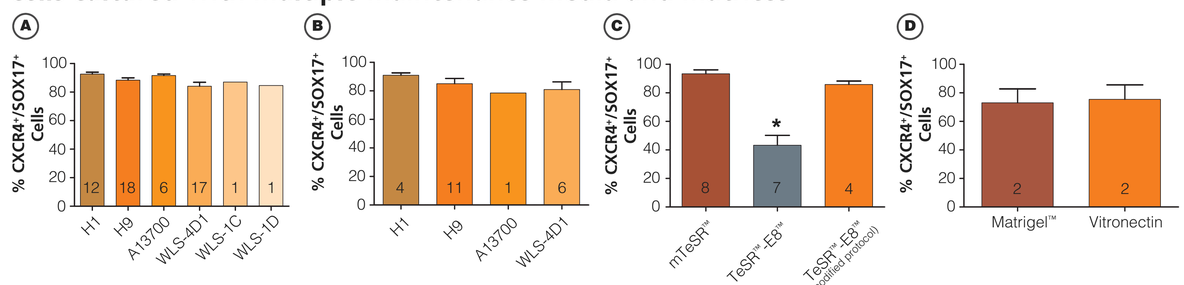
Representative dot plots showing CXCR4 and SOX17 co-expression in hES cells (H1 and H9) and hiPS cells (WLS-4D1 and A13700) following 5 days of differentiation using STEMdiff™ Definitive Endoderm. Isotype controls were used to set quadrant gates.

FIGURE 2: Differentiation is accompanied by loss of pluripotency markers and gain of definitive endoderm markers



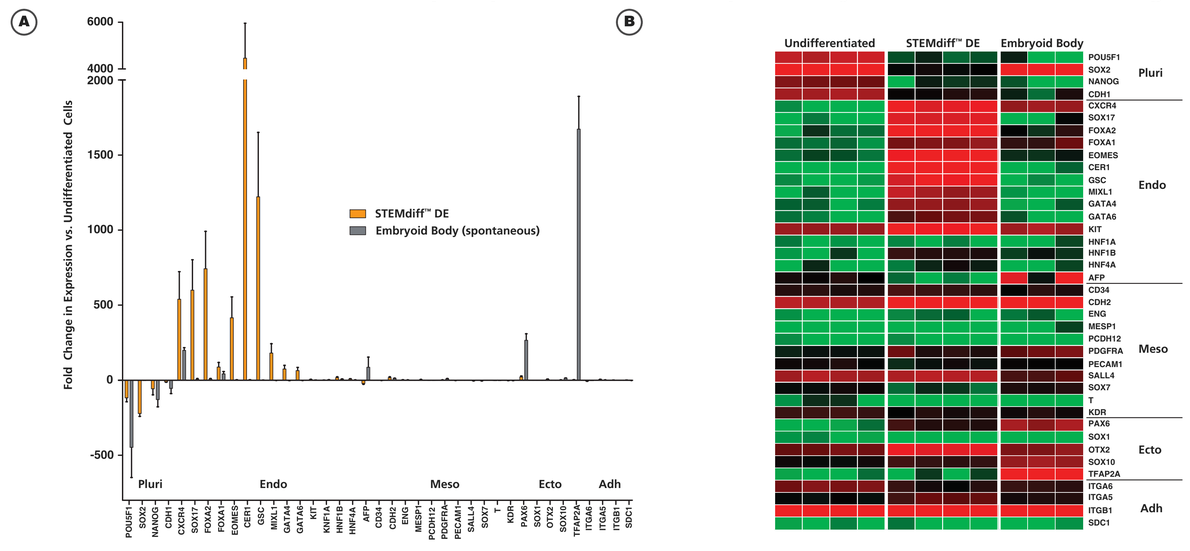
A) Quantitative analysis of undifferentiated (Ai, H9; Bi, WLS-4D1) and Day 5 differentiated (Aii, H9; Bii, WLS-4D1) human pluripotent stem cells indicates a down-regulation of the pluripotency and anterior foregut endoderm marker SOX2 with a concomitant up-regulation in the definitive endoderm markers SOX17 and FOXA2. C) Quantitative analysis of marker expression at daily intervals during differentiation of H9 cells to definitive endoderm reveals up-regulation of DE markers (CXCR4/SOX17) by Day 3 and a loss of pluripotency markers (OCT-4/SSEA-3) by Day 4. Transient expression of PDGFR-α on Day 3 suggests a transition through a mesendoderm state. D) Verification of SOX17/FOXA2 expression by immunocytochemistry. Scale bars = 100 µm.

FIGURE 3: Definitive endoderm formation is efficient when using human pluripotent stem cells cultured with multiple maintenance media and matrices



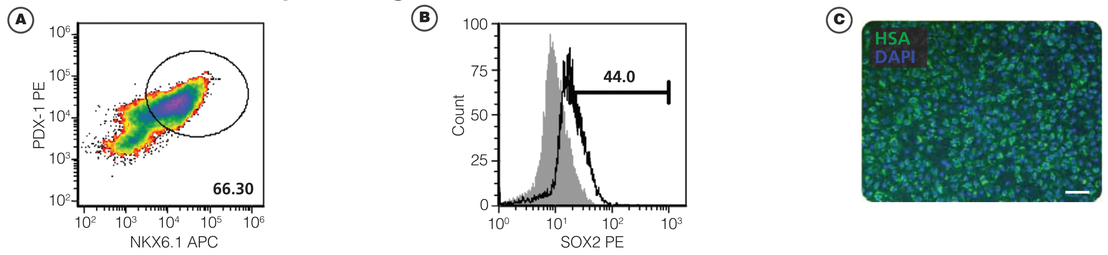
Quantitative analysis of CXCR4/SOX17 co-expression in multiple differentiated hES and hiPS cell lines previously cultured in A) mTeSR™1, B) TeSR™2, or C) TeSR™-E8™. For high efficiency DE differentiation of TeSR™-E8™ cultures, protocol optimization was required (C; *P < 0.05 vs. mTeSR™1). D) Definitive Endoderm differentiation of human pluripotent stem cells maintained in TeSR™2 is equally efficient when using human recombinant vitronectin as a matrix as compared to Matrigel™, thus providing a completely defined, animal protein-free culture system for definitive endoderm formation. Data are expressed as mean ±SEM; n values are indicated by the number within each bar.

FIGURE 4: Genome-wide expression profile indicates that STEMdiff™ Definitive Endoderm directs differentiation of human pluripotent stem cells specifically to the endoderm lineage



A) Expression pattern of key pluripotency (Pluri), endoderm (Endo), mesoderm (Meso), and ectoderm (Ecto) lineage markers and cell adhesion (Adh) genes of H9 hES cells are shown. Data were acquired using the Illumina HumanHT-12 v4 BeadChip and are expressed as relative fold change in expression compared to undifferentiated H9 cells (n = 3 - 4 for each group). Human pluripotent stem cells were either formed into embryoid bodies and differentiated spontaneously for 5 days in the presence of 10% serum or directed to definitive endoderm using the STEMdiff™ Definitive Endoderm Kit. B) A heat map representation of the data depicted in A. Highly expressed genes are shown in red, genes with minimal expression are shown in green.

FIGURE 5: STEMdiff™ Definitive Endoderm yields DE that is capable of downstream differentiation to multiple lineages



Following differentiation to definitive endoderm using the STEMdiff™ Definitive Endoderm Kit, cells were further differentiated using published protocols to pancreatic progenitors (Rezania et al., Diabetes 2012), pulmonary progenitors (Wong et al., Nature Biotechnology, 2012), or hepatocyte progenitors (Hay et al., Stem Cells, 2008). A) Representative flow cytometry analysis of PDX-1 and NKX6.1 co-expression (circled) following differentiation of H9-derived definitive endoderm to pancreatic progenitors. B) Representative flow cytometry analysis of SOX2 expression following differentiation of WLS-4D1-derived definitive endoderm to pulmonary progenitors. Isotype control is shown in grey and was used to set the gate. C) Representative image depicting human serum albumin (HSA) immunoreactivity of hepatocyte progenitors following differentiation of H9-derived definitive endoderm. Scale bars = 100 µm.

Summary

- STEMdiff™ Definitive Endoderm is a fully defined, animal component-free medium formulation that allows for reproducible differentiation of hES and hiPS cells to definitive endoderm.
- Differentiation is highly 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 pulmonary, pancreatic, and hepatic lineages.
- Efficiency of differentiation to the endoderm lineage using STEMdiff™ Definitive Endoderm is equally efficient with human pluripotent stem cells maintained in mTeSR™1, TeSR™2, or TeSR™-E8™ using Matrigel™ or human recombinant vitronectin as a matrix.
- STEMdiff™ Definitive Endoderm can reliably be used as a starting point for studies aimed at the formation of endoderm cell lineages from hES and hiPS cells.