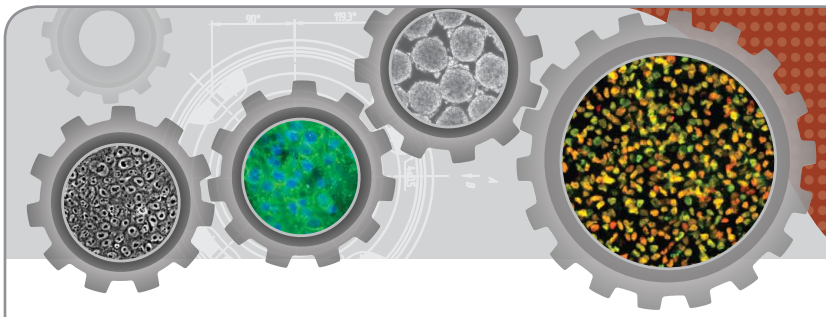

STEMdiff™ Definitive Endoderm Kit

Easy, Standardized hPSC Differentiation to Definitive Endoderm Cells

The **STEMdiff™ Definitive Endoderm Kit** is a defined, animal component-free system that enables the differentiation of human pluripotent stem cells (hPSCs) to multipotent definitive endoderm cells, using a short and simple protocol (Figure 1). This product is available in formulations optimized for use with hPSCs cultured in mTeSR™1 or TeSR™-E8™. hPSCs differentiated using either method are highly enriched for definitive endoderm cells, as indicated by co-expression of SOX17, CXCR4 and FOXA2 (Figures 2-4). Furthermore, differentiation is efficient and reproducible across multiple human embryonic stem (hES) and induced pluripotent stem (hiPS) cell lines (Figure 2). Definitive endoderm cells generated with this kit can be further differentiated to multiple downstream endodermal cell types, including hepatic and pancreatic progenitor cells (Figure 5) for drug development, toxicity testing, research for development of cell-based therapies, or studying developmental pathways.

Advantages:

ANIMAL COMPONENT-FREE. Defined, serum- and animal component-free formulation.

OPTIMIZED. Compatible with hPSCs cultured in mTeSR™1 or TeSR™-E8™.

ROBUST. Reproducible differentiation of multiple human ES and iPS cell lines.

MULTIPOTENT. Generate functional endoderm capable of downstream differentiation to multiple lineages.

PRODUCT	CAPACITY	CATALOG #
STEMdiff™ Definitive Endoderm Kit	1 kit	05110
STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™-Optimized)	1 kit	05115
EasySep™ Human CXCR4 Positive Selection Kit	For labeling up to 1 x 10^9 cells	18163

Short and Simple Protocol

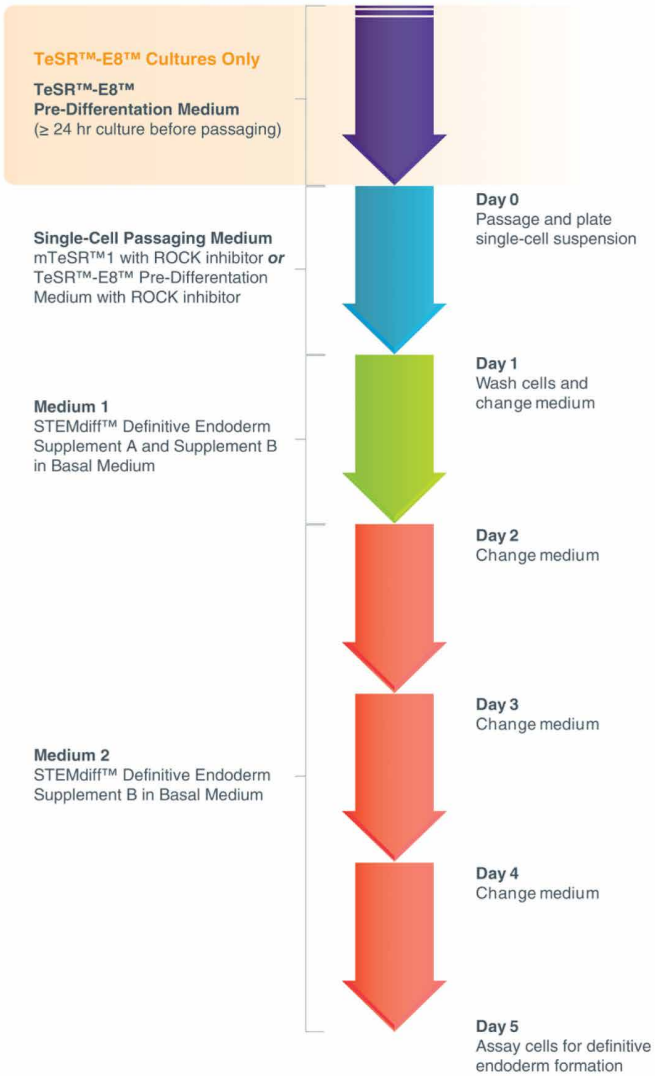

Figure 1. Differentiation Protocol

STEMdiff™
Definitive Endoderm Kit

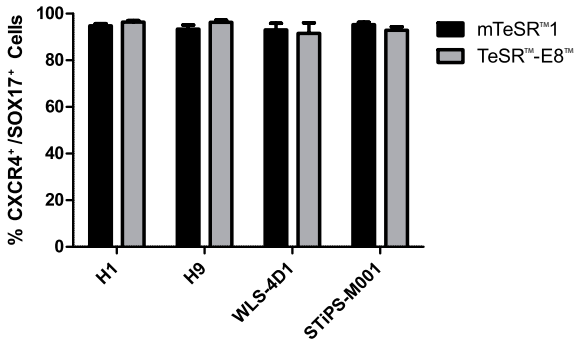

Figure 2. Definitive Endoderm Differentiation is Efficient Across Multiple hES and hiPS Cell Lines, Regardless of hPSC Maintenance Medium

Quantitative analysis of definitive endoderm formation in multiple hES (H1 and H9) and hiPS (WLS-4D1 and STiPS-M001) cell lines as measured by co-expression of CXCR4 and SOX17. Cells maintained in mTeSR™1 medium were differentiated using the STEMdiff™ Definitive Endoderm Kit, and cells maintained in TeSR™-E8™ were differentiated using the STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™-Optimized). Data are expressed as the mean percentage of cells expressing both markers. Error bars indicate SEM; n = 4 to 18 per cell line.

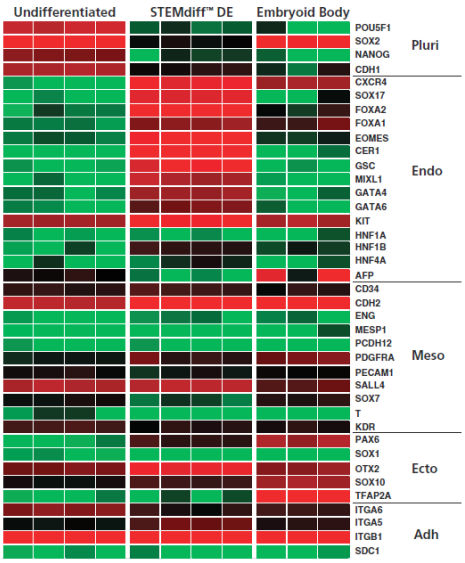

Figure 3. The STEMdiff™ Definitive Endoderm Kit Directs Differentiation Specifically to the Endoderm Lineage

Heat map expression data 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 Human HT-12 v4 BeadChip. Highly expressed genes are shown in red; genes with minimal expression are shown in green. hPSCs were maintained in mTeSR™1 and then either formed into embryoid bodies and differentiated spontaneously for 5 days in the presence of 10% serum, or directed to definitive endoderm cells using the STEMdiff™ Definitive Endoderm Kit.

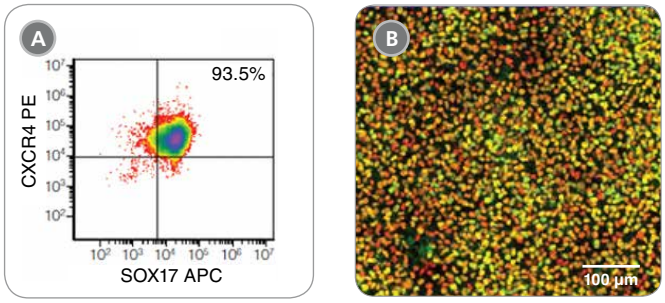

Figure 4. Efficient Expression of Key Definitive Endoderm Markers in hPSCs Differentiated with the STEMdiff™ Definitive Endoderm Kit

(A) Representative density plot showing CXCR4 and SOX17 expression in mTeSR™1-cultured H1 hES cells, following 5 days of differentiation. (B) Representative image of FOXA2 (green) and SOX17 (red) in WLS-4D1 hiPS cells following 4 days of differentiation. Yellow indicates cells co-expressing FOXA2 and SOX17.

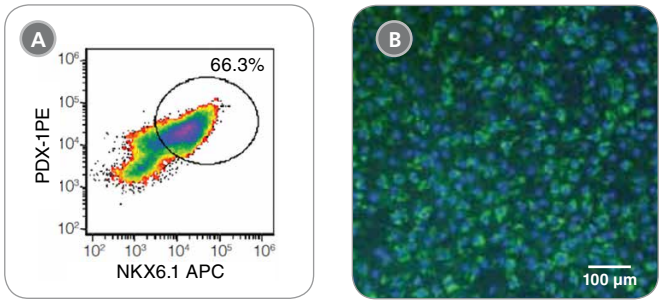

Figure 5. The STEMdiff™ Definitive Endoderm Kit Yields Definitive Endoderm Cells that are Capable of Downstream Differentiation to Multiple Lineages

Following generation of definitive endoderm using the STEMdiff™ Definitive Endoderm Kit, cells were further differentiated using published protocols to pancreatic[1] or hepatic progenitors.[2] (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 image depicting human serum albumin (HSA; green) immunoreactivity of hepatic progenitors following differentiation of H9-derived definitive endoderm cells.

References

1. Rezania A, et al. (2012) Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 61(8):2016-29.
2. Hay DC, et al. (2008) Efficient differentiation of hepatocytes from human embryonic stem cells exhibiting markers recapitulating liver development in vivo. Stem Cells 26(4):894-902.

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit **www.stemcell.com/DEworkflow** or contact us at **techsupport@stemcell.com**.