STEMdiff™ Mesoderm Induction Medium

Defined and Xeno-Free Medium for Differentiation to Early Mesoderm

Simple Differentiation to Early Mesoderm Cells

STEMdiff™ Mesoderm Induction Medium (MIM) is a defined, xeno-free medium for generation of early mesoderm cells from human embryonic stem (ES) and induced pluripotent stem (iPS) cells. Protocols for mesodermal differentiation can be difficult and inconsistent, therefore, use the short and simple STEMdiff™ MIM monolayer protocol (Figure 1) to start off your human pluripotent stem cell (hPSC) differentiation by generating quality early mesoderm.

STEMdiff™ MIM produces a cell population enriched for early mesoderm, as indicated by positive expression of Brachyury (T) and NCAM and lack of OCT4 expression (Figures 2-4). As part of the hPSC workflow, STEMdiff™ MIM is compatible with hPSCs cultured in TeSR™ media, and efficiently differentiates hPSCs cultured in either mTeSR™1 or TeSR™-E8™ (Figure 3). When directed, early mesoderm cells produced using STEMdiff™ MIM can be further differentiated to specialized cell types, such as mesenchymal stem cells and their derivatives (osteoblasts, chondrocytes, adipocytes) and endothelial cells (Figures 5,6).

PRODUCT	SIZE	CATALOG #
STEMdiff™ Mesoderm Induction Medium	100 mL	05220
	500 mL	05221

Advantages of STEMdiff™ MIM:

- Defined and xeno-free
- Rapid induction of mesoderm after only 2 - 4 days of differentiation
- Efficient and reproducible differentiation of multiple human ES and iPS cell lines
- Generates early mesoderm cells that are capable of differentiation to multiple downstream cell types

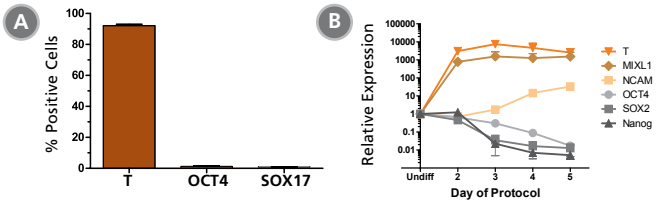

FIGURE 2. STEMdiff™ MIM Generates a Homogenous Population of T^+OCT4^- Early Mesoderm

(A) Data showing marker expression characteristic of the early mesoderm (positive Brachyury (T) expression and negative OCT4 and SOX17 expression) on day 5 of the protocol. Data is expressed as a mean percentage of cells expressing each marker ± SD, n = 33 (T, OCT4); n = 5 (SOX17). **(B)** Expression of undifferentiated cell markers (OCT4, SOX2, NANOG) and early mesoderm markers (T, MIXL1, NCAM), measured by quantitative PCR (qPCR) and normalized to levels in undifferentiated cells; n = 2.

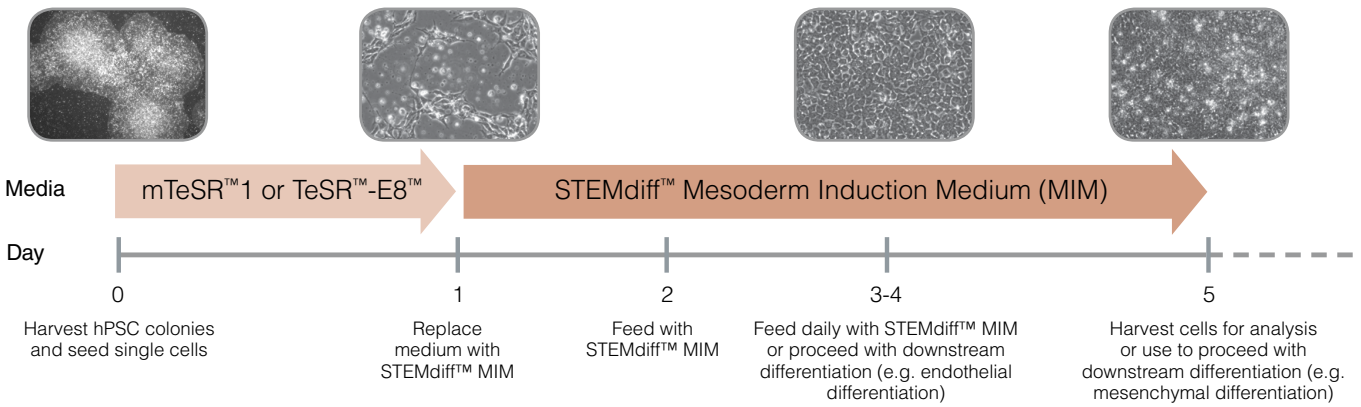

FIGURE 1. Schematic of Mesoderm Induction Medium Differentiation Timeline

On day 0, hPSC colonies are harvested and seeded as single cells at 5 x 10^4 cells/cm^2 in mTeSR™1 or TeSR™-E8™ and supplemented with 10 µM Y-27632. TeSR™ medium is replaced on day 1 with STEMdiff™ Mesoderm Induction Medium when cells are at approximately 20 - 50% confluency. Cells are then fed daily and cultured in STEMdiff™ MIM (days 2-4). Cells can be transferred to downstream differentiation conditions between days 3 - 5 or collected on day 5 for analysis.

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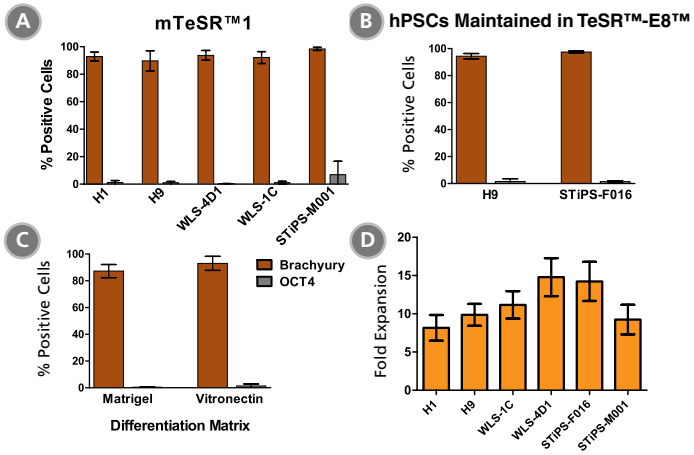

FIGURE 3. Mesoderm Differentiation and Cell Expansion are Efficient and Comparable Across Multiple hPSC Cell Lines

Graphs show mesoderm formation in multiple human ES (H1 and H9) and iPS (WLS-4D1, WLS-1C, STiPS-M001 and STiPS-F016) cell lines as measured by expression of Brachyury (T) and absence of OCT4. Cells maintained in **(A)** mTeSR™1 or **(B)** TeSR™-E8™ medium were differentiated using STEMdiff™ MIM. (A; n = 2 - 10 per cell line; B; n = 3; data are expressed as a mean percentage ± SD) **(C)** Mesoderm differentiation on Corning® Matrigel® or Vitronectin XF™ is comparable. (n = 5; data are the mean percentage ± SD) **(D)** Average fold expansion of cells cultured in STEMdiff™ MIM, as determined by cell yield / cells seeded. (n = 3 - 13. Error bars indicate SEM)

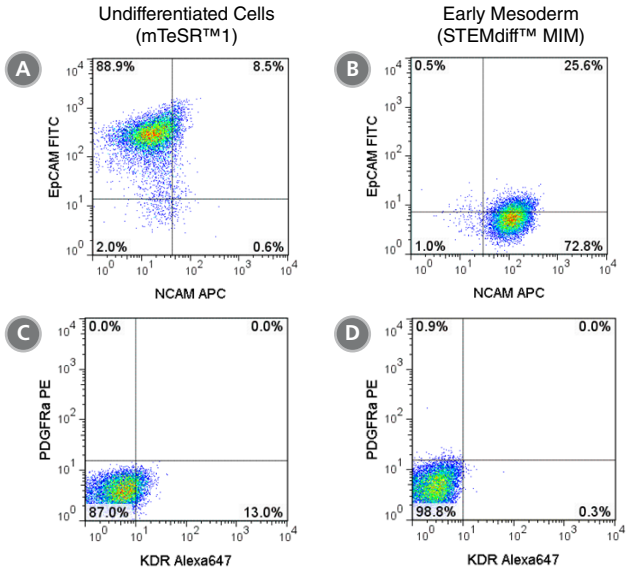

FIGURE 4. Phenotype of Cells Treated with STEMdiff™ MIM is Consistent with Early Mesoderm

Representative flow cytometry plots showing the switch from **(A)** EpCAM^{+}NCAM$^{-/low}$ in hPSCs cultured in mTeSR™1 to **(B)** EpCAM$^{-/low}$NCAM^{+} expression in STEMdiff™ MIM-treated cells (day 5). EpCAM$^{-/low}$NCAM^{+} expression is characteristic of the early mesoderm. Expression of PDGFRα and KDR are low in both **(C)** hPSCs cultured in mTeSR™1 and **(D)** early mesoderm cells derived with STEMdiff™ MIM.

Downstream Differentiation

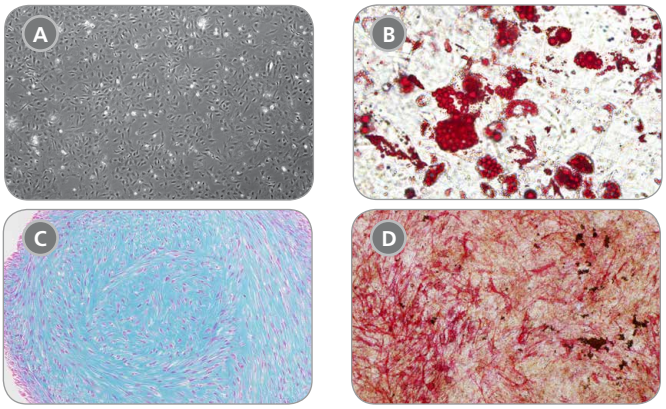

FIGURE 5. Mesenchymal Stem Cells Derived from Early Mesoderm Cells Can Be Further Differentiated in In Vitro Assays

(A) Early mesoderm cells generated with the 5-day STEMdiff™ MIM protocol and subsequently cultured with MesenCult™-ACF develop mesenchymal stem cell (MSC)-like morphology, 40X magnification. MSC-like cells can subsequently differentiate into **(B)** adipocytes (Oil Red O staining), 200X magnification; **(C)** chondrocytes (Alcian Blue staining), 100X magnification; and **(D)** osteogenic cells (Fast Red and Silver Nitrate staining), 40X magnification.

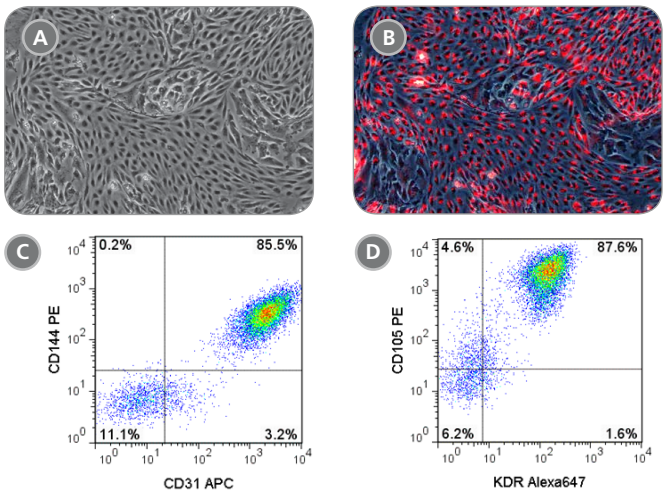

FIGURE 6. Robust Endothelial Differentiation of STEMdiff™ MIM-Generated Early Mesoderm Cells

On day 3 of the STEMdiff™ MIM protocol, early mesoderm cells were switched to a downstream endothelial differentiation protocol based on Tan et al.[1] **(A)** Differentiated cells display characteristic endothelial cell morphology and **(B)** are able to uptake Dil-Ac-LDL (red). Representative flow cytometry plots showing **(C)** 85.5% CD144^{+}CD31^{+} and **(D)** 87.6% CD105^{+}KDR^{+} expression in differentiated endothelial cells.

References

1. Tan JY, et al. (2013) Stem Cells Dev 22(13): 1893-1906

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