Highly efficient differentiation of human pluripotent stem cells maintained in TeSR™-E8™ to definitive endoderm

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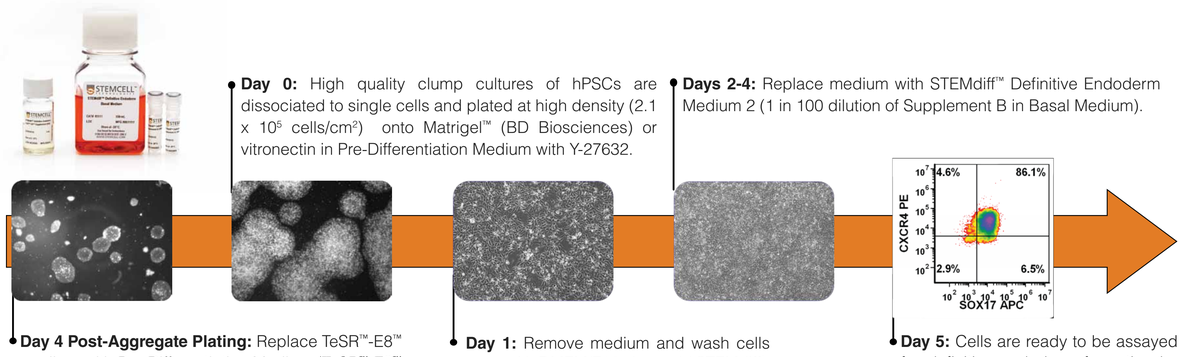
Introduction

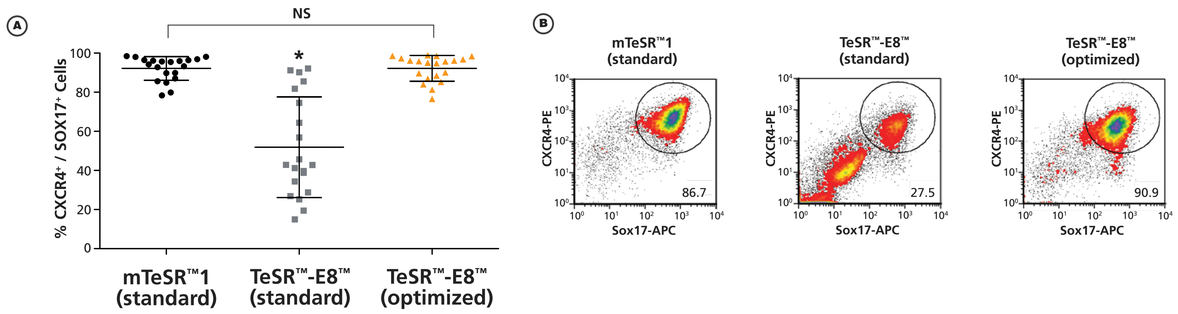
The formation of definitive endoderm (DE) from human pluripotent stem cells (hPSCs) is required for development of specialized cells of endoderm organs. Recent studies show that hPSCs can differentiate to liver, pancreas, lung, and intestinal cell types. Critical in these processes is the initial development of a highly pure population of DE. In addition, the eventual translation of laboratory protocols towards clinical applications requires defined culture systems. TeSR™-E8™ is a simplified, xeno-free, and low-protein maintenance medium for culturing undifferentiated hPSCs. However, DE differentiation is less efficient in hPSCs cultured in TeSR™-E8™ compared to mTeSR™1 when using our animal component-free (ACF) STEMdiff™ Definitive Endoderm differentiation kit. To continue to meet the growing need for robust differentiation protocols that function across multiple cell lines maintained in these newly-developed maintenance culture conditions we developed STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized), an ACF medium that promotes the highly efficient differentiation of multiple hPSC lines to functional DE. Cells differentiated using this optimized medium and protocol show similar marker expression to hPSCs previously maintained in mTeSR™1 and are capable of efficient downstream differentiation towards the pancreatic lineage. Down-regulation of markers of the undifferentiated state and up-regulation of endoderm markers occur within a similar timeframe compared to cells previously maintained in mTeSR™1, suggesting that DE generated from this optimized differentiation kit is similar to that generated from mTeSR™1 cultures. Both ACF STEMdiff™ Definitive Endoderm kits provide a method of reproducible and highly efficient generation of DE from hPSCs, whether using mTeSR™1 or TeSR™-E8™.

FIGURE 1: Product format and protocol



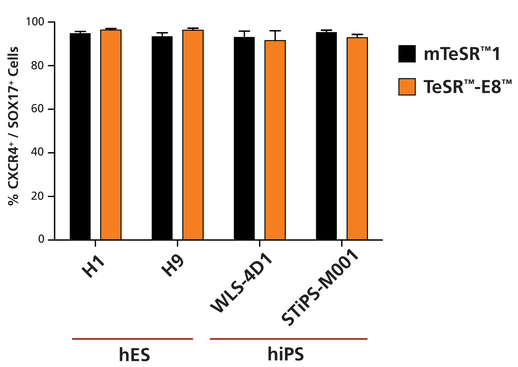
Results

FIGURE 2: Optimized media and protocols are required for efficient definitive endoderm differentiation from TeSR™-E8™



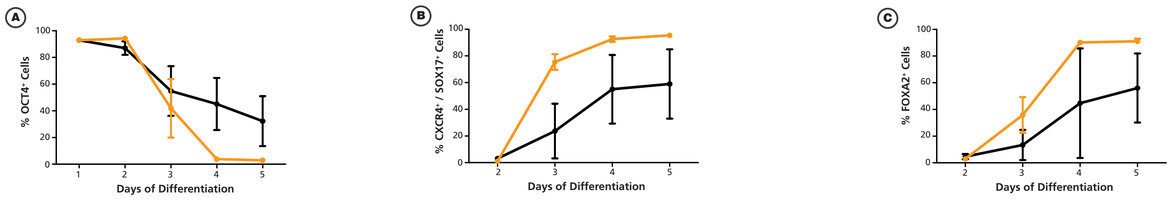
(A) Cumulative data showing CXCR4/SOX17 co-expression in human embryonic stem (hES) cell lines (H1 and H9) and human induced pluripotent stem (hiPS) cell lines (WLS-4D1 and STiPS-M001) maintained in mTeSR™1 or TeSR™-E8™ and differentiated to DE using the standard STEMdiff™ DE Kit or the new TeSR™-E8™ Optimized DE Kit. Cells maintained in mTeSR™1 differentiate with high efficiency and low variability. Cells maintained in TeSR™-E8™ also exhibit high efficiency and low variability but only if differentiated using the new optimized medium and protocol (individual data points are represented by dots; error bars indicate the mean ± SD; n = 20; *p<0.05 vs. mTeSR™1; NS = not significant). **(B)** Representative flow cytometry dot plots showing examples of average CXCR4/SOX17 co-expression in H1 cells maintained in mTeSR™1 (left) or TeSR™-E8™ and differentiated using the standard STEMdiff™ DE Kit (middle) or cells maintained in TeSR™-E8™ and differentiated using the TeSR™-E8™ Optimized Kit (right).

FIGURE 3: STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized) promotes efficient DE differentiation in multiple hPSC lines



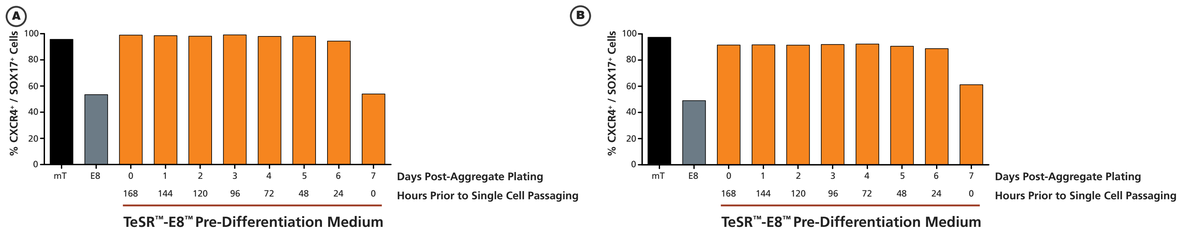
Quantitative analysis of CXCR4/SOX17 co-expression in hPSCs previously maintained in either mTeSR™1 (black) or TeSR™-E8™ (orange) and differentiated using either the standard (black) or TeSR™-E8™ Optimized (orange) STEMdiff™ DE Kit (n = 4 - 18 per cell line). No significant differences were observed between cultures of the same cell line as assessed by paired t-test.

FIGURE 4: Differentiation of TeSR™-E8™ cultures to definitive endoderm is accompanied by loss of pluripotency markers and up-regulation of definitive endoderm markers



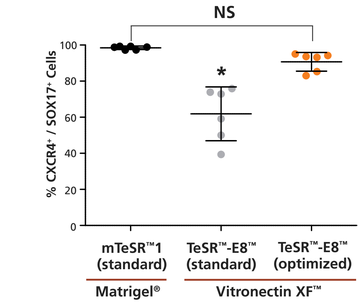
(A) Down-regulation of OCT4 is delayed and expression remains higher at day 5 in TeSR™-E8™ cultures differentiated with the standard STEMdiff™ DE Kit (black) compared to cultures differentiated with the TeSR™-E8™ Optimized Kit (orange). **(B,C)** Up-regulation of endoderm markers CXCR4 and SOX17 **(B)** and FOXA2 **(C)** is delayed and peaks at a lower value in TeSR™-E8™ cultures differentiated with the standard STEMdiff™ DE Kit (black) compared to cultures differentiated with the TeSR™-E8™ Optimized Kit (orange). Data were generated from H1 and STiPS-M001 cells and are shown as the mean ± SEM; n = 3.

FIGURE 5: Cells maintained in TeSR™-E8™ require at least 48 hours of pre-differentiation to achieve highly efficient differentiation to definitive endoderm



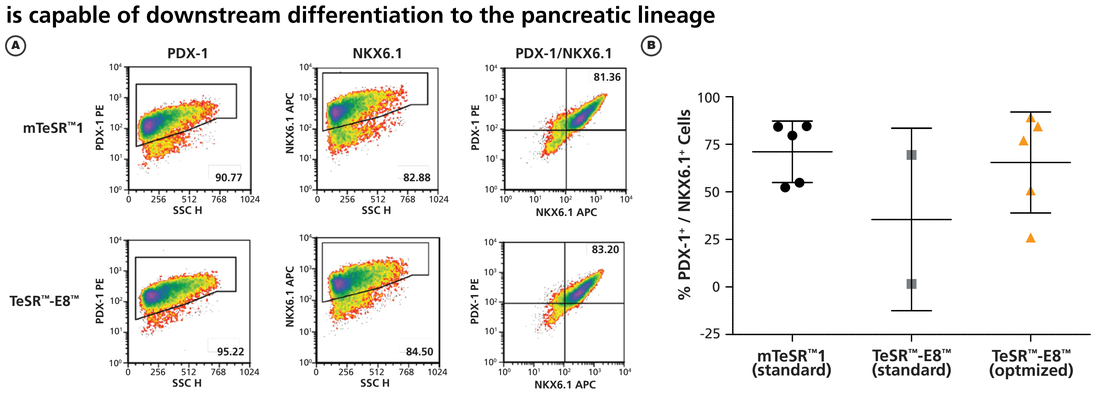
Quantitative analysis of CXCR4/SOX17 co-expression in **A)** H1 hES cells or **B)** STiPS-M001 hiPS cells maintained in TeSR™-E8™ and differentiated to DE. Undifferentiated cells were maintained on a 7-day passaging schedule. Cells maintained in mTeSR™1 and differentiated using the standard STEMdiff™ DE Kit serve as a positive control (black). Cells maintained in TeSR™-E8™ and differentiated using the standard STEMdiff™ DE Kit serve as a negative control (gray). Test cultures (orange) were treated with the novel pre-differentiation medium either at the time of aggregate plating (0 days post-aggregate plating) or at the beginning of each day after aggregate plating. The same data are also displayed in hours prior to generating a single cell suspension for initiating DE differentiation (Figure 1, Day 0). Performance is high if cells are pre-treated for at least 24 hours and up to 7 days prior to generation of a single-cell suspension. Data plotted as the mean of two experiments.

FIGURE 6: A xeno-free workflow can be achieved using hPSCs maintained and differentiated on Vitronectin XF™



H1 or STiPS-M001 cells maintained in mTeSR™1 on Matrigel® (black) differentiate with high efficiency. These same cell lines maintained in TeSR™-E8™ on Vitronectin XF™ (gray) show similar variability and a significant reduction in differentiation efficiency to cells that are maintained in TeSR™-E8™ on Matrigel®. Using the TeSR™-E8™ Optimized Kit, cells maintained in TeSR™-E8™ on Vitronectin XF™ (orange) differentiate with high efficiency and low variability (individual data points are represented by dots; error bars indicate the mean ± SD; n = 6 for each condition; *p <0.001 verus the two other conditions; NS = not significant).

FIGURE 7: STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized) yields functional definitive endoderm that is capable of downstream differentiation to the pancreatic lineage



H1 hES cells were maintained in mTeSR™1 or TeSR™-E8™ prior to differentiation using either the standard or TeSR™-E8™ Optimized STEMdiff™ DE Kit. DE cells were then differentiated towards pancreatic precursor cells using a published protocol (Schulz *et al.*, PLoS One, 2012). Differentiation efficiency was assessed by flow cytometric analysis of PDX-1 and NKX6.1 co-expression. **(A)** Representative flow cytometry dot plots show efficient pancreatic precursor differentiation in H1 cells. **(B)** Cumulative quantitative data for PDX-1/NKX6.1 co-expression for cells maintained in mTeSR™1 or TeSR™-E8™ (individual data points are represented by dots; error bars indicate the mean ± SD; n = 2 - 6 for each condition).

Summary

- STEMdiff™ Definitive Endoderm Kits promote reproducible and highly efficient differentiation of hPSCs to DE.
- Optimization of the medium and protocol was required to ensure robust performance from hPSCs maintained in TeSR™-E8™.
- TeSR™-E8™ cultured cells require at least 48 hours of pre-differentiation to achieve highly efficient DE differentiation.
- The new STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized) Kit is compatible with hPSCs cultured and differentiated on Matrigel® or Vitronectin XF™.
- DE cells derived from TeSR™-E8™ cultures is capable of further specification towards downstream lineages.