TeSR™-E7™

Reprogramming Medium for Human iPS Cell Induction

Reproducible generation of iPS cells with high-quality colony morphology for easy identification and rapid subcloning

Human induced pluripotent stem (iPS) cells are generated by reprogramming somatic cells to a pluripotent state, usually via temporary over-expression of select reprogramming factors. Traditionally, reprogramming conditions have included mouse embryonic fibroblast (MEF) feeder layers and undefined media containing animal-derived components. This leads to variable efficiencies[1,2] and iPS cell colonies that are difficult to identify and select.

TeSR™-E7™ is an animal component-free (ACF) reprogramming culture medium optimized for the generation of human iPS cells without the use of feeders. It is based on the E7 formulation published by the laboratory of Dr. James Thomson (University of Wisconsin-Madison).[3] TeSR™-E7™ is intended for use during the induction phase of reprogramming (Figure 1) and can be used with Corning® Matrigel® hESC-qualified matrix (Matrigel®), or, for a completely xeno-free system, Vitronectin XF™ (developed and manufactured by Primorigen Biosciences). The medium is optimized to limit fibroblast overgrowth, resulting in colonies with easily recognizable embryonic stem (ES)-cell like morphology.

Advantages:

EASY TO IDENTIFY AND SELECT COLONIES
Pre-screened components ensure high quality iPS cell colony morphology for improved manual selection.

RAPID SUBCLONING
Reduced differentiation and fibroblast growth enables rapid establishment of homogeneous iPS cell cultures.

REPRODUCIBLE EFFICIENCY
Feeder-free, animal component-free formulation facilitates reproducibly efficient human iPS cell generation.

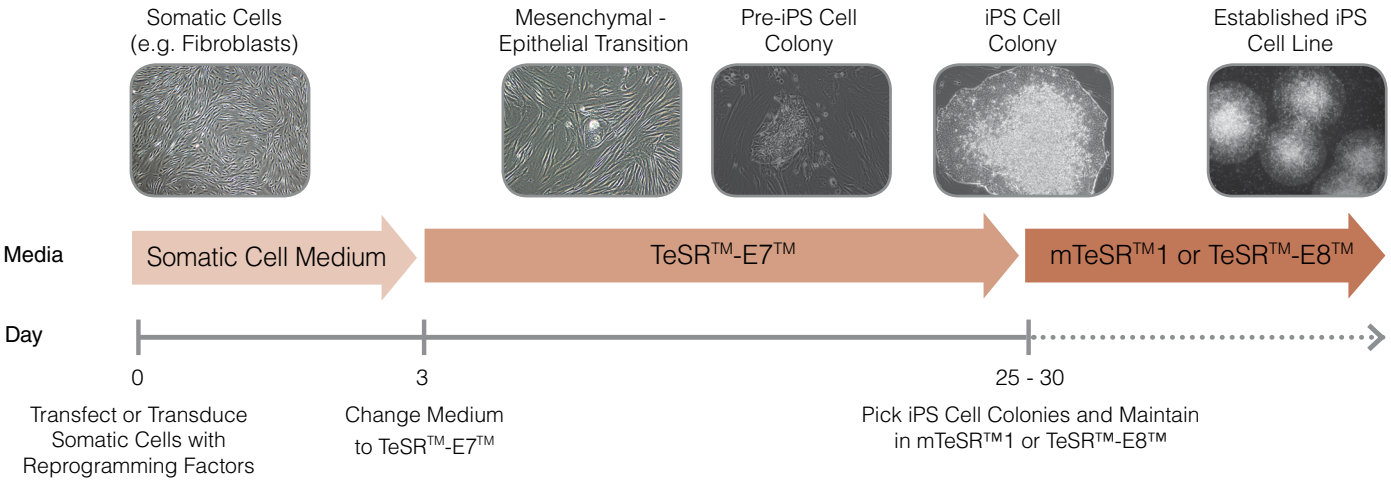


FIGURE 1. Schematic of reprogramming timeline

TeSR™-E7™ can be used during the entire induction phase of reprogramming (day 3 to 25+). Following reprogramming, iPS cell colonies can be isolated and propagated in feeder-free maintenance systems (eg. mTeSR™1 or TeSR™-E8™ media on Matrigel® or Vitronectin XF™ matrices).

Easy to Identify and Select Colonies

Primary iPS cell colonies generated in TeSR™-E7™ medium are easily recognizable by their characteristic ES cell-like morphology. The morphological changes that occur during the induction phase of the reprogramming process are shown in Figure 2. By 3-4 weeks, colonies with typical ES cell-like morphology including defined colony borders and tightly packed cells can be observed (Figure 2E,F). The similarity of these primary colonies to those in established feeder-free cultures makes them easy to identify for clonal selection and expansion.

Colonies generated in TeSR™-E7™ have fewer areas of differentiation and less fibroblast overgrowth compared to commonly used culture conditions such as KnockOut™ Serum Replacement (KOSR)-based iPS cell induction medium (Figure 3). This further adds to their ease of isolation, since fibroblasts and differentiated areas do not need to be removed from the colony prior to selection. These morphological advances are due to both the absence of non-essential components (e.g. TGFβ), and the pre-screening of key components in TeSR™-E7™, such as bFGF (Figure 4).

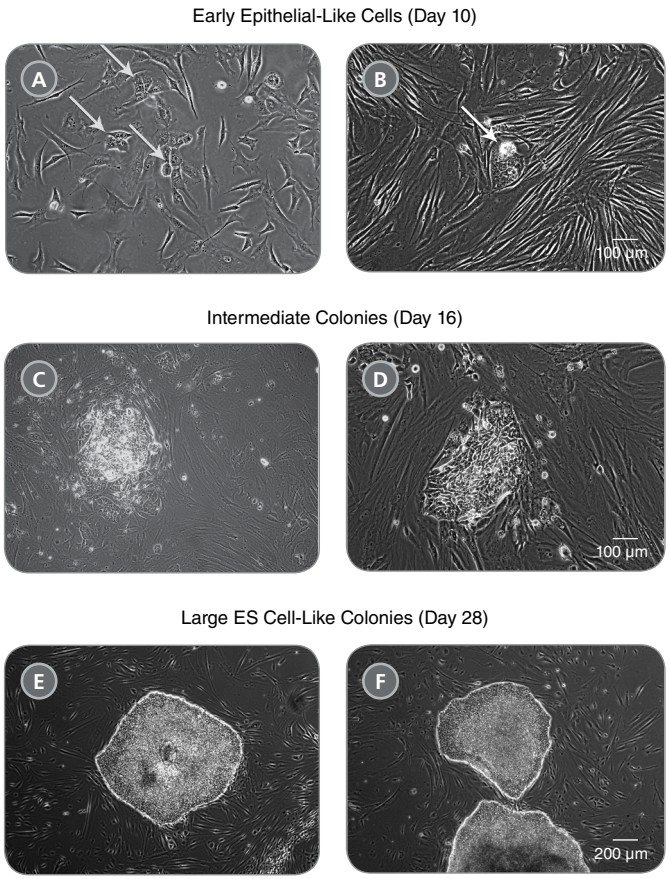


FIGURE 2. Morphology of representative iPS cell colonies arising during the induction period in TeSR™-E7™

(**A,B**) Small clusters of colonies with an epithelial-like morphology will appear by one to two weeks following induction (see arrows). (**C,D**) These clusters expand into pre-iPS cell colonies by two to three weeks. (**E,F**) Larger ES cell-like colonies are clearly identifiable by three to four weeks. Representative colonies from adult human fibroblasts reprogrammed with episomal vectors containing OCT-4, SOX2, KLF-4, and L-MYC are shown.

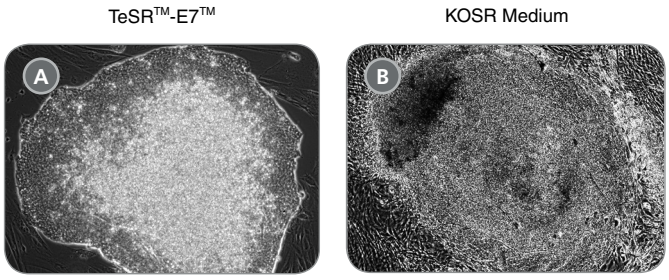


FIGURE 3. Comparison of primary iPS cell colonies derived using TeSR™-E7™ and KOSR-based medium

(**A**) TeSR™-E7™ generates colonies with defined borders and less overgrowth of background fibroblasts compared to (**B**) KOSR-based iPS cell induction medium. Representative colonies from adult human fibroblasts reprogrammed with episomal vectors containing OCT-4, SOX2, KLF-4, and L-MYC are shown.

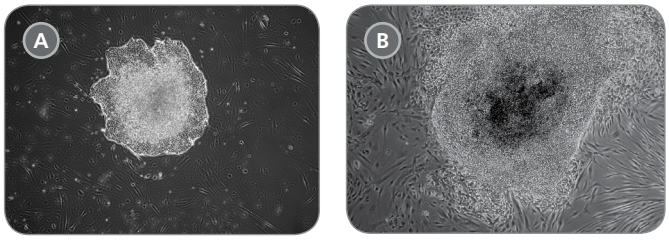
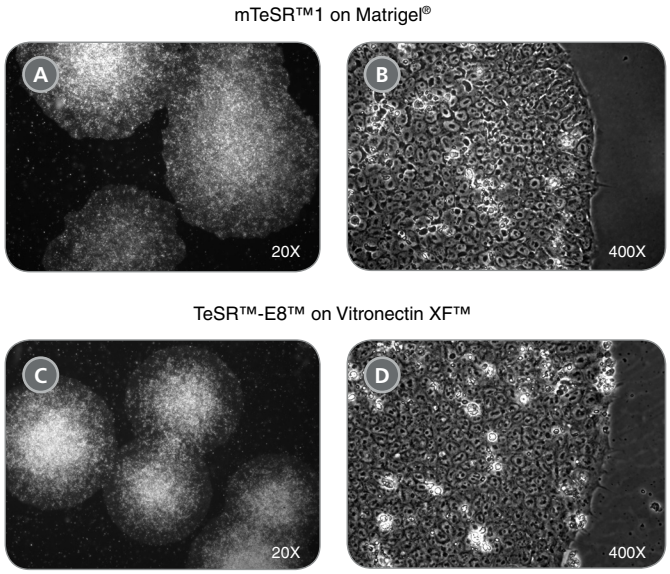


FIGURE 4. Comparison of primary iPS cell colonies derived using TeSR™-E7™ with qualified vs. unqualified bFGF

(**A**) TeSR™-E7™ yields easily recognizable iPS cell colonies with defined borders. (**B**) Unqualified components can result in colonies that have poorly defined edges and higher levels of differentiation. Representative colonies from adult human fibroblasts reprogrammed with episomal vectors containing OCT-4, SOX2, KLF-4, and L-MYC are shown.

Rapid Subcloning

The high quality of iPS cell colonies generated in TeSR™-E7™ means highly undifferentiated cell lines can be generated as early as passage 2 when expanded in feeder-free maintenance media, such as mTeSR™1 or animal component-free (ACF) TeSR™-E8™ (Figure 5). Unlike iPS cell lines generated and maintained in feeder-dependent or other undefined conditions, fewer passages of manual selection are required to establish cultures with low levels of differentiation.



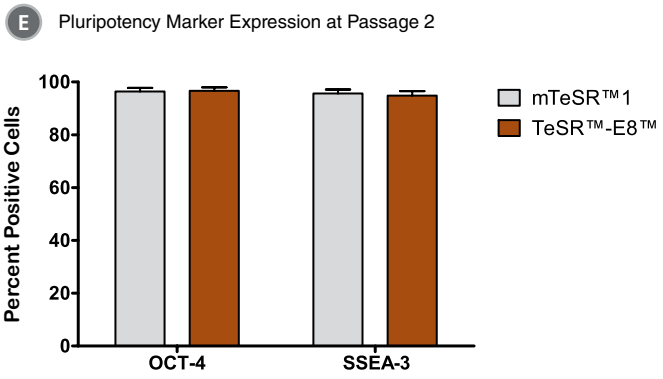


FIGURE 5. iPS cell colonies expanded in mTeSR™1 or TeSR™-E8™

(**A-D**) iPS cell colonies generated in TeSR™-E7™ and expanded in either mTeSR™1 on Matrigel® (A,B) or TeSR™-E8™ on Vitronectin XF™ (C,D) exhibit classic ES cell morphology with dense colony centers, defined borders, prominent nucleoli and high nuclear-to-cytoplasmic ratios. (**E**) iPS cells express high levels of pluripotency markers after just two passages in either mTeSR™1 or TeSR™-E8™ as demonstrated by OCT-4 and SSEA-3 flow cytometry analysis. Data are expressed as mean ± SEM, n = 4.

Reproducible Efficiency

The reprogramming efficiency with TeSR™-E7™ is equivalent to or greater than that with KOSR-based iPS cell induction medium as demonstrated using an episomal reprogramming system on fibroblast cell lines (Figure 6). The reprogramming efficiency is also higher with TeSR™-E7™ than with serum-free maintenance media, such as TeSR™-E8™, due to the absence of TGFβ (a MET inhibitor). Other cell types such as bone marrow-derived mesenchymal stromal cells and CD34+ hematopoietic progenitor cells have also been successfully reprogrammed in TeSR™-E7™ (data not shown). TeSR™-E7™ has been optimized for use with a feeder-free system, avoiding the inherent batch-to-batch variability of feeder cells[1] that leads to inconsistent reprogramming efficiencies.

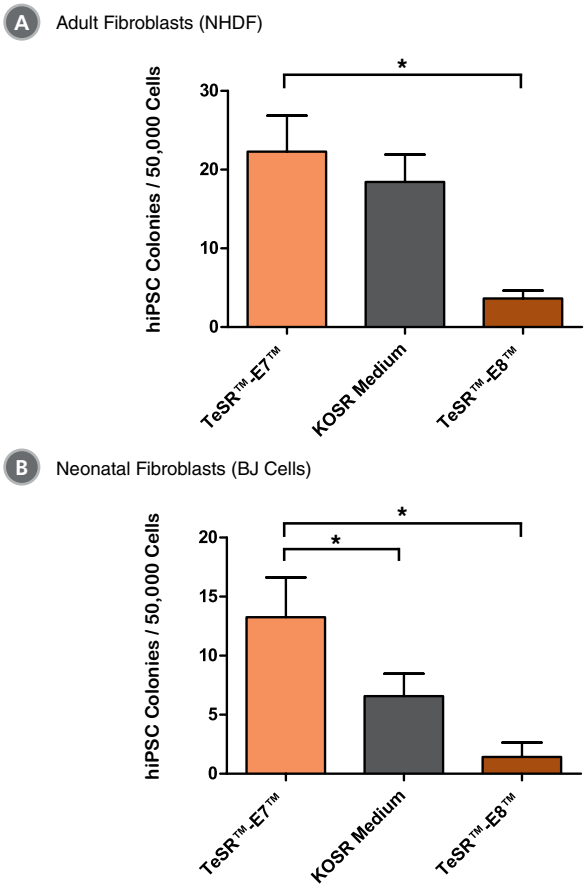


FIGURE 6. TeSR™-E7™ supports reprogramming of human cell types including adult dermal fibroblasts and neonatal fibroblasts

(**A,B**) Reprogramming of adult normal human dermal fibroblasts (NHDF, 33 year-old female; A) and BJ neonatal foreskin fibroblasts (B) with episomal reprogramming vectors are shown. TeSR™-E7™ demonstrated similar, in NHDF, or greater, in BJ fibroblasts, reprogramming efficiencies compared to KOSR-based iPS cell induction medium. TeSR™-E7™ demonstrated higher reprogramming efficiencies compared to TeSR™-E8™. Data are expressed as mean ± SEM, n ≥ 6, * p ≤ 0.05.

TeSR™-E7™

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Bona Fide iPS Cell Lines

iPS cell lines derived in TeSR™-E7™ and maintained in mTeSR™1 or TeSR™-E8™ show high pluripotent marker expression (Figure 5), normal karyotype (Figure 7), and are able to differentiate into cells of all 3 germ layers (Figure 8).

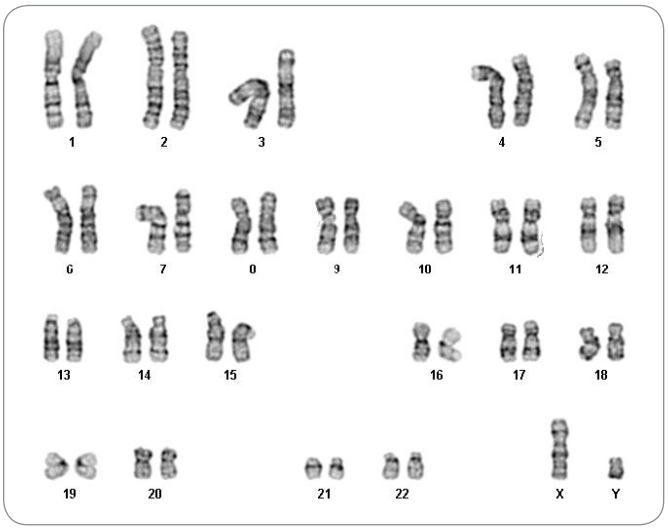

FIGURE 7. iPS cells derived in TeSR™-E7™ display normal karyotype

iPS cell lines were generated in TeSR™-E7™ medium, maintained in mTeSR™1 or TeSR™-E8™ media for a minimum of 5 passages and karyotyped by G-banding karyotype analysis. Three iPS cell lines were analyzed and all demonstrated a normal karyotype, a representative karyogram is shown.

PRODUCT	SIZE	CATALOG #
TeSR™-E7™	500 mL	05914
mTeSR™1	500 mL	05850
TeSR™-E8™	500 mL	05940
Vitronectin XF™	1 kit	07190
STEMdiff™ Definitive Endoderm Kit	1 kit	05110
STEMdiff™ Neural Induction Medium	1 kit	05835
STEMdiff™ APEL™2 Medium	100 mL	05270

TABLE 1. Products for human iPS cell derivation, maintenance, and differentiation

PRIMORIGEN BIOSCIENCES®
the stem cell company™

To functionally assess pluripotency of TeSR™-E7™-derived iPS cell lines, directed differentiations to cell types of all three germ layers were performed (Figure 8). TeSR™-E7™-derived iPS cells were expanded in mTeSR™1 and differentiated to endoderm using STEMdiff™ Definitive Endoderm Kit, to mesoderm using STEMdiff™ APEL™ Medium and a published protocol[4], and to ectoderm using STEMdiff™ Neural Induction Medium.

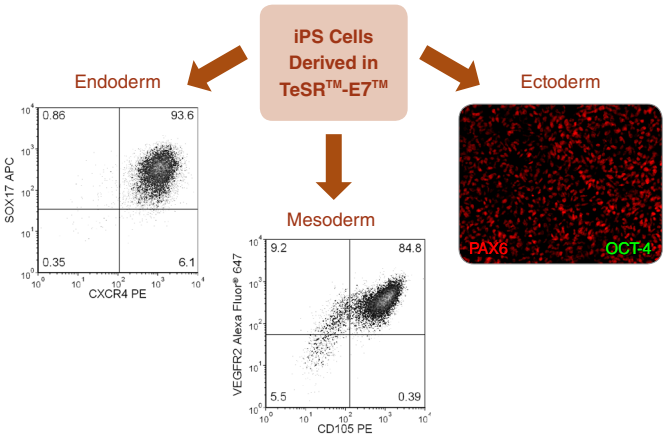

FIGURE 8. Directed differentiation of iPS cells to all three germ layers

TeSR™-E7™-derived iPS cells were differentiated into all three germ layers. Endoderm specification was achieved using the STEMdiff™ Definitive Endoderm Kit; results demonstrated 93.6% SOX17$^+$CXCR4$^+$ cells. Mesoderm specification was demonstrated using a STEMdiff™ APEL™ Medium-based endothelial differentiation protocol; results demonstrated >99% CD31$^+$ cells (data not shown) and 84.8% VEGFR2$^+$CD105$^+$ cells. Ectoderm specification was demonstrated using STEMdiff™ Neural Induction Medium; immunocytochemistry shows high levels of PAX6 staining with no detectable OCT-4 staining by day 9 of neural induction.

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

References

1. Zou C et al. (2012) Efficient derivation and genetic modifications of human pluripotent stem cells on engineered human feeder cell lines. Stem Cells Dev 21(12): 2298-311.
2. Ludwig TE and Thomson JA. (2007) Defined culture media for human embryonic stem cells. Embryonic Stem Cells (The Netherlands: Springer): 1-16.
3. Chen G et al. (2011) Chemically defined conditions for human iPSC derivation and culture. Nat Methods 8(5): 424-429.
4. Tan JY. (2013) Efficient derivation of lateral plate and paraxial mesoderm subtypes from human embryonic stem cells through GSKi-mediated differentiation. Stem Cells Dev 22(13): 1893-1906.