TeSR™-E7™

Feeder-free and animal component-free reprogramming medium for human iPS cell induction

Catalog #05914 500 mL Kit

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Product Description

TeSR™-E7™ is a serum-free, low-protein, and animal component-free medium that was originally developed for reprogramming fibroblasts with episomally delivered reprogramming vectors.[1] TeSR™-E7™ has a similar formulation to TeSR™-E8™ (Catalog #05990), with the removal of TGF-β, to reduce fibroblast overgrowth and promote mesenchymal-to-epithelial transition. TeSR™-E7™ is intended for cellular reprogramming of human somatic cells (e.g. fibroblasts) to induced pluripotent stem (iPS) cells.

This medium may be used with either Corning® Matrigel® hESC-Qualified Matrix (Corning Catalog #354277) or Vitronectin XF™ (Catalog #07180, a matrix developed and manufactured by Nucleus Biologics) as the culture matrix.

Product Information

The following components are sold as a complete kit (Catalog #05914) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
TeSR™-E7™/ReproTeSR™ Basal Medium	05919	480 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
TeSR™-E7™ 25X Supplement	05915	20 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

Please refer to the Safety Data Sheet (SDS) for hazard information.

Preparation of Complete TeSR™-E7™

Use sterile technique to prepare complete TeSR™-E7™ (Basal Medium + 25X Supplement). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

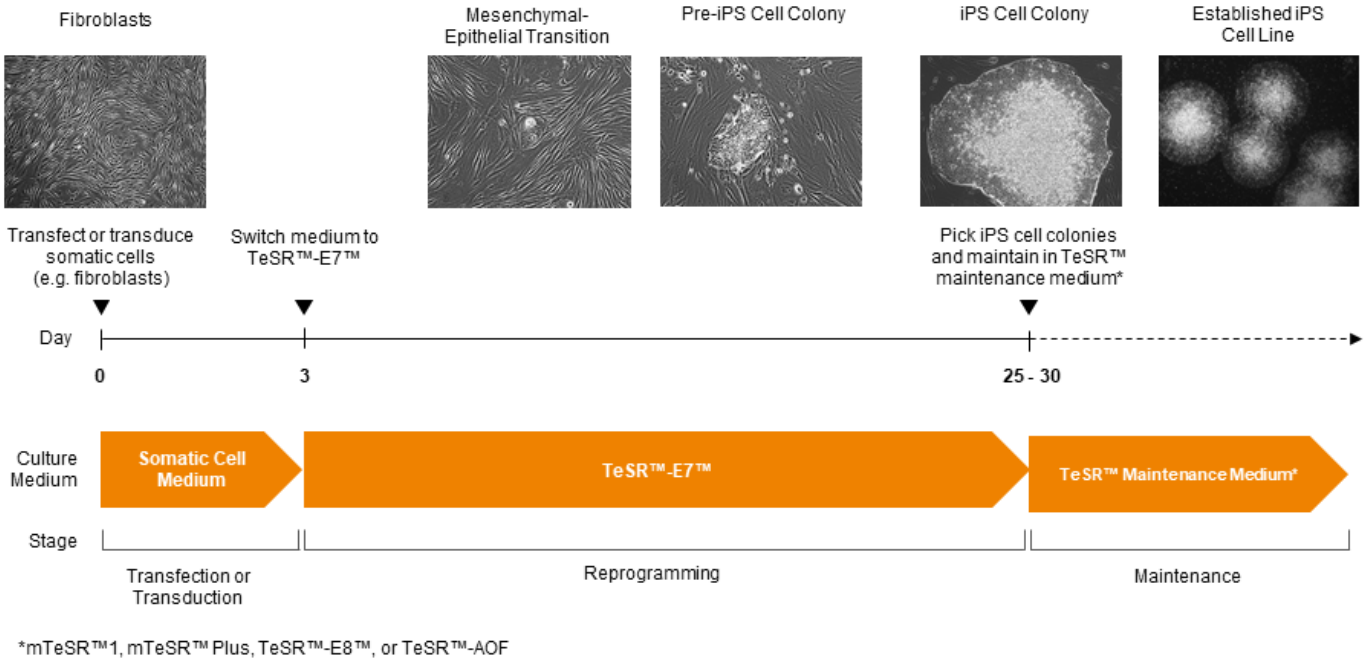
1. Thaw 25X Supplement at room temperature (15 - 25°C) or at 2 - 8°C just prior to use. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.

2. Add 20 mL of 25X Supplement and to 480 mL of Basal Medium. Mix thoroughly.

   NOTE: If prepared aseptically, complete TeSR™-E7™ is ready for use and does not require filtering. If not used immediately, store complete TeSR™-E7™ at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C for up to 1 month. Do not exceed the shelf life of the individual components. Thaw complete TeSR™-E7™ at room temperature or overnight at 2 - 8°C. Once thawed, use medium within 1 week. Do not re-freeze.

Reprogramming Time Course



*mTeSR™1, mTeSR™ Plus, TeSR™-E8™, or TeSR™-AOF

Directions for Use

Please read the entire protocol before proceeding.

Indicated volumes are for a single well of a 6-well tissue culture-treated plate (e.g. Catalog #38015). If using alternative cultureware, adjust volumes accordingly.

1. On **Day 0**, transfect or transduce somatic cells using desired reprogramming vector system.

   NOTE: Transfection/transduction protocol should be optimized for each vector system and cell type. For a detailed example refer to the Technical Bulletin: Reprogramming Human Dermal Fibroblasts in TeSR™-E7™ to Induced Pluripotent Stem Cells Using an Episomal Vector System (Document #28065), available at www.stemcell.com or contact us to request a copy.

2. Plate transfected/transduced cells onto a 6-well plate coated with desired matrix, such as Corning® Matrigel® or Vitronectin XF™.

   NOTE: For complete instructions on coating plates with Corning® Matrigel® or Vitronectin XF™, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #10000005505), available at www.stemcell.com or contact us to request a copy.

3. On **Day 1**, aspirate medium and add 2 mL of medium specific to the somatic cell type being reprogrammed. Incubate at 37°C for 48 hours.

4. On **Day 3**, aspirate medium and add 2 mL of complete TeSR™-E7™. Incubate at 37°C for 24 hours.

5. Perform daily medium changes (2 mL/well) using complete TeSR™-E7™. Monitor the cells until iPS cell colonies appear.

   NOTE: iPS cell colonies typically arise between days 20 - 30 but may vary depending on cell type and vector system used. To achieve optimal reprogramming efficiency, it is recommended to use somatic cells at low passage. For a representative example of an iPS cell colony, refer to the figure.

   NOTE: It is acceptable once per week to double-feed the cells (i.e. add 4 mL of complete TeSR™-E7™ per well) and skip a medium change the following day during the first 2 weeks of reprogramming.

6. Manually isolate putative iPS cell colonies as described below.

   NOTE: If there are many untransfected, partially reprogrammed, and/or differentiated cells surrounding the putative iPS cell colony, these may need to be scraped away prior to isolating the iPS cell colony.

   a. Use either a 22 - 25 gauge needle or a pulled glass pipette to cut the putative iPS cell colony into small fragments.

   b. Use a 200 µL pipettor with a filtered pipette tip to scrape and remove colony fragments.

7. Immediately plate iPS cell colony fragments on cultureware coated with desired matrix (e.g. Corning® Matrigel® or Vitronectin XF™) and containing TeSR™ maintenance medium (e.g. mTeSR™1, mTeSR™ Plus, TeSR™-E8™, or TeSR™-AOF).

   NOTE: To facilitate the initial attachment of iPS cell colony fragments, add Y-27632 (Catalog #72302) to the maintenance medium at a final concentration of 10 µM. After 24 hours, replace the maintenance medium (without Y-27632).

8. Incubate at 37°C and perform medium changes as required.

NOTE: For complete instructions on maintaining iPS cells using mTeSR™1, mTeSR™ Plus, TeSR™-E8™, or TeSR™-AOF, refer to the corresponding Technical Manuals in the table below. Documents are available at www.stemcell.com or contact us to request a copy.

PRODUCT	CATALOG #	DOCUMENT #
mTeSR™1	85850/85857/85870/85875	10000005505
mTeSR™ Plus	100-0276	10000007757
TeSR™-E8™	05990	10000005516
TeSR™-AOF	100-0401	10000008450

References

1. Chen G et al. (2011) Chemically defined conditions for human iPSC derivation and culture. Nat Methods 8(5): 424–9.