TeSRTM-E7TM

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

Catalog #05914 500 mL Kit



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Product Description

TeSRTM-E7TM is a serum-free, low-protein, and animal component-free medium that was originally developed for reprogramming fibroblasts with episomally delivered reprogramming vectors. TeSRTM-E7TM has a similar formulation to TeSRTM-E8TM (Catalog #05990), with the removal of TGF- β , to reduce fibroblast overgrowth and promote mesenchymal-to-epithelial transition. TeSRTM-E7TM 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 12 months from date of manufacture (MFG) on label.

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

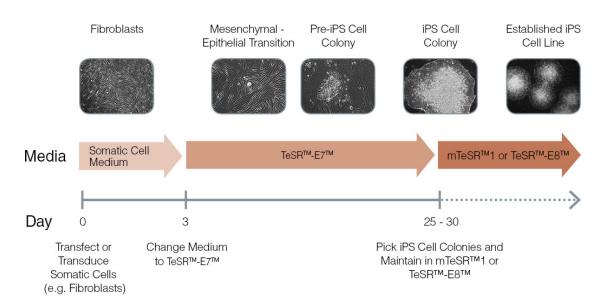
Preparation of Complete TeSR™-E7™ Medium

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

- 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 TeSRTM-E7TM medium is ready for use and does not require filtering. If not used immediately, store complete TeSRTM-E7TM medium 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 TeSRTM-E7TM medium at room temperature (15 25°C) or overnight at 2 8°C. Once thawed, use medium within 1 week. Do not re-freeze.



Reprogramming Time Course



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 TeSRTM-E7TM 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™ medium. 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 TeSR TM -E7 TM 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 follows:
 - 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 micropipette with a filtered pipette tip to scrape and aspirate colony fragments.
 - 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.
- 7. Immediately plate iPS cell colony fragments on cultureware coated with desired matrix (e.g. Corning® Matrigel® or Vitronectin XFTM) and containing iPS cell maintenance medium (e.g. mTeSRTM1 or TeSRTM-E8TM).
 - 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 iPS cell maintenance medium changes accordingly.

NOTE: For complete instructions on how to maintain iPS cells using mTeSRTM1 or TeSRTM-E8TM, 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
TeSR™-E8™	05990	10000005516

References

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



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