ReproTeSR™

Blood reprogramming medium for human iPS cell induction

Catalog #05920 500 mL Kit

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Product Description

ReproTeSR™ is a complete, xeno-free and defined reprogramming medium developed for generating human induced pluripotent stem (iPS) cells from blood-derived cells such as CD34+ or erythroid precursor cells under feeder-free conditions.

This medium is recommended for use during the induction phase of reprogramming blood-derived cells and may be used with Corning® Matrigel® hESC-Qualified Matrix (Corning Catalog #354277).

Easy to identify and select colonies

- Pre-screened components ensure high quality of iPS cell colony morphology for improved manual selection

Rapid subcloning

- Large colony size and reduced differentiation enable rapid establishment of homogeneous iPS cell cultures

Reproducible efficiency

- Feeder-free, defined formulation facilitates reproducibly efficient human iPS cell generation

Product Information

The following components are sold as a complete kit (Catalog #05920) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
ReproTeSR™ Basal Medium	05921	474 mL	Store at 2 - 8°C.	Stable for 18 months from date of manufacture (MFG) on label.
ReproTeSR™ 20X Supplement	05922	25 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
ReproTeSR™ 500X Supplement	05923	1 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 ReproTeSR™ Medium

Use sterile techniques when preparing complete ReproTeSR™ medium (Basal Medium + 20X Supplement + 500X Supplement). The following example is for preparing 500 mL of ReproTeSR™ medium. If preparing other volumes, adjust accordingly.

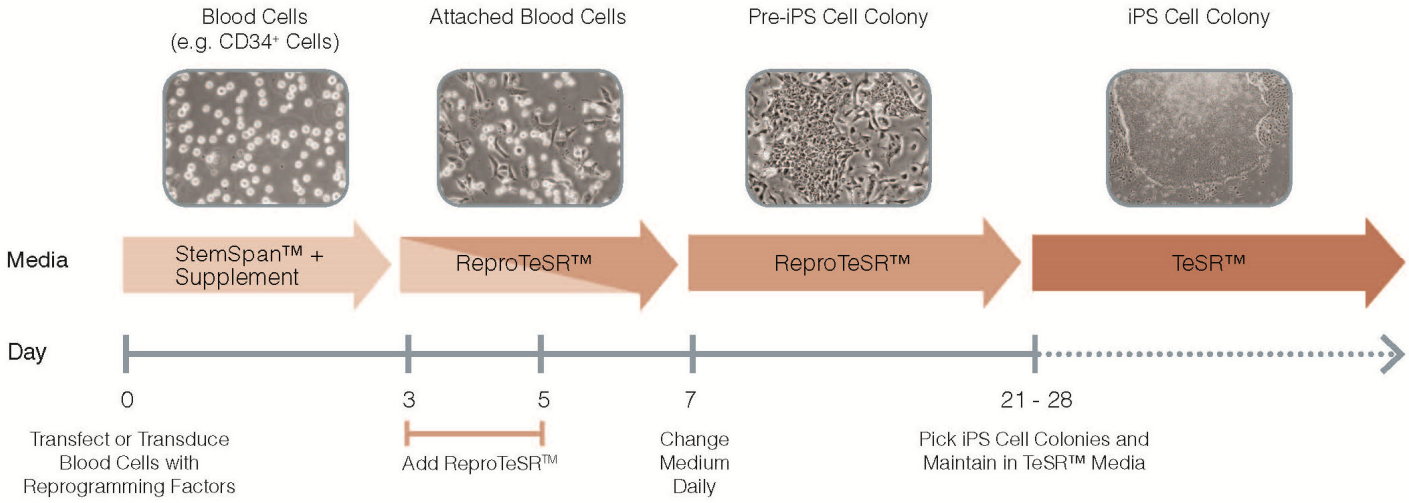
1. Thaw ReproTeSR™ 20X Supplement and ReproTeSR™ 500X Supplement at room temperature (15 - 25°C) or at 2 - 8°C.

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

2. Add 25 mL of ReproTeSR™ 20X Supplement and 1 mL of ReproTeSR™ 500X Supplement to 474 mL of ReproTeSR™ Basal Medium. Mix thoroughly.

   NOTE: If prepared using sterile techniques, complete ReproTeSR™ medium is ready for use and does not require filtering. If not used immediately, store complete ReproTeSR™ 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. After thawing complete medium, use immediately or store at 2 - 8°C for up to 2 weeks. 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 plate. If using other cultureware, adjust volumes accordingly. Prior to reprogramming, the isolation and culture expansion of blood-derived cells such as CD34+ or erythroid precursor cells may be required. For further information, refer to www.stemcell.com.

1. On **Day 0**, transfect or transduce blood-derived 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: TeSR™-E7™ Episomal Protocol (Document #28065) available at www.stemcell.com or contact us to request a copy.

2. Transfer cells to a 15 mL conical tube (e.g. Catalog #38009) containing blood cell growth medium. Resuspend cells at a density of 75,000 - 150,000 cells per mL of growth medium.

   NOTE: Examples of blood cell growth media include StemSpan™ SFEM II (Catalog #09605) in combination with either of the following:

   - StemSpan™ CD34+ Expansion Supplement (10X; Catalog #02691)
   - StemSpan™ Erythroid Expansion Supplement (100X; Catalog #02692)

   For further information, refer to www.stemcell.com.

3. Plate 150,000 - 300,000 cells/well (i.e. 2 mL cell suspension/well) onto Matrigel®-coated wells of a 6-well plate.

   NOTE: The suggested plating density is optimized for transfecting CD34+ cells with an episomal system. Plating density may need further optimization depending on the vector system used and growth kinetics of the cells being reprogrammed.

4. On **Day 2**, add 1 mL of the same blood cell growth medium (without removing any medium from the well). Incubate at 37°C.
5. On **Day 3**, add 1 mL of ReproTeSR™ medium (without removing medium from the well). Incubate at 37°C.
6. On **Day 5**, add 1 mL of ReproTeSR™ medium (without removing medium from the well). Incubate at 37°C.
7. On **Day 7**, aspirate medium from each well and replace with 2 mL of fresh ReproTeSR™ medium. Incubate at 37°C.

   NOTE: Attachment of cells to matrix with epithelial-like or mesenchymal-like features is typically seen by Day 7. This represents the early reprogramming phase of blood-derived cells.

8. Perform daily medium changes (2 mL/well) using ReproTeSR™ medium. Monitor the cells until iPS cell colonies appear.

   NOTE: iPS cell colonies typically arise between Days 21 and 28 but may vary depending on cell type, vector system used, and transfection/transduction efficiency. See the figure for an overview of the reprogramming time course.

9. 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.
10. Immediately plate iPS cell colony fragments on cultureware coated with desired matrix (e.g. Corning® Matrigel®) and containing iPS cell maintenance medium (e.g. mTeSR™1 [Catalog #85850] or TeSR™-E8™ [Catalog #05940]).

    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).
11. Incubate at 37°C and perform iPS cell maintenance medium changes accordingly.

    NOTE: For complete instructions on how to maintain iPS cells using mTeSR™1 or TeSR™-E8™, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315) or TeSR™-E8™ (Document #29267), available at www.stemcell.com or contact us to request a copy.