

# TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium

Cat. No. MP101

Storage: at the proper storage temperature for one year

## Description

TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium is a complete, chemically defined and xeno-free growth medium for the expansion of human pluripotent stem cells (hPSCs) under feeder-free conditions. The formulation is modified for the robust proliferation of hPSCs in the undifferentiated state.

## Kit Contents & Storage

| Component                                                                        | MP101-01 | Storage                                               |
|----------------------------------------------------------------------------------|----------|-------------------------------------------------------|
| TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Basal Medium | 500 ml   | at 2-8°C                                              |
| TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Supplement   | 5×1 ml   | at -20°C in the dark<br>avoid repeated freeze-thawing |

## Procedures

Materials required but not included

| Product Name                                                         | Catalog                    |
|----------------------------------------------------------------------|----------------------------|
| 0.5 mM EDTA (in PBS without calcium or magnesium)                    |                            |
| PBS without calcium or magnesium                                     | TransGen, Cat. FG701-01    |
| Y-27632                                                              | TransGen, Cat. MS101-01    |
| TransStem™ Chemically Defined Xeno-free Cell Cryopreservation Medium | TransGen, Cat. MC101-01    |
| Vitronectin                                                          | Thermo Fisher, Cat. A14700 |

### 1. Prepare Complete TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium

Add 1 ml of thawed TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Supplement to 99 ml of TransStem™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Basal Medium. Mix thoroughly.

### 2. Coat culture vessels with vitronectin

Dilute sufficient amounts of thawed vitronectin to a final concentration of 5 µg/ml with PBS at room temperature, resuspend gently. Add sufficient amounts of vitronectin dilution to culture vessels (Table 1). Incubate the coated vessels at 37°C for 1 hour or at 2-8°C overnight. Do not allow the vessel to dry. Aspirate and discard the vitronectin solution before use.

Table 1 Reagent volumes (in ml per well or per dish)

| Culture vessel | Approx. surface area        | Volume of diluted vitronectin solution |
|----------------|-----------------------------|----------------------------------------|
| 6-well plate   | 10 cm <sup>2</sup> per well | 1.0 ml                                 |
| 12-well plate  | 4 cm <sup>2</sup> per well  | 0.5 ml                                 |
| 24-well plate  | 2 cm <sup>2</sup> per well  | 0.25 ml                                |
| 35-mm dish     | 10 cm <sup>2</sup>          | 1.0 ml                                 |
| 60-mm dish     | 20 cm <sup>2</sup>          | 2.0 ml                                 |
| 100-mm dish    | 60 cm <sup>2</sup>          | 6.0 ml                                 |

### 3. Passage human PSCs

When hPSC colonies are large enough, or cover approximately 90% of the surface area of the culture vessel, hPSC colonies can be typically split 1:6 to 1:10. The exact split ratio can vary for each cell line.

- (1) Remove media from hPSC cultures and wash once with DMEM/F12.
- (2) Add enough 0.5 mM EDTA to cover the entire surface area of each well then incubate at 37°C for 3 minutes.
- (3) Once cell colonies have detached from each other but not from the culture vessel, carefully aspirate the EDTA solution, and wash once with DMEM/F12.
- (4) Add appropriate amount of Complete *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium to resuspend the detached colonies gently, and plate cell aggregate mixture at the desired density onto pre-coated culture vessels with vitronectin.

### 4. Cryopreserve human PSCs

HPSCs maintained in *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium can be cryopreserved using *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Cell Cryopreservation Medium.

- (1) Remove media from hPSC cultures and wash once with DMEM/F12.
- (2) Add enough 0.5 mM EDTA to cover the entire surface area of each well then incubate at 37°C for 3 minutes.
- (3) Once cell colonies have detached from each other but not from the culture vessel, carefully aspirate the EDTA solution, and wash once with DMEM/F12.
- (4) Add appropriate amount of DMEM/F12 to resuspend the detached colonies gently. Transfer the detached cell aggregates to a 15 ml conical tube. Centrifuge cells at 400×g for 3 minutes, then remove supernatant.
- (5) Resuspend the cell pellet in cold cryopreservation medium at the recommended viable cell density. Dispense aliquots of the cell suspension into cryogenic vials. It is recommended to freeze around 1-2×10<sup>6</sup> cells per cryogenic vial. One well of a 6-well plate gives rise to approximately 1-2×10<sup>6</sup> cells.
- (6) Immediately place cryogenic vials in a controlled freezing container. Store the controlled freezing container containing cryogenic vials at -80°C overnight. Transfer frozen cells to liquid nitrogen for long term storage.

Note: Ensure cryopreservation medium and the controlled freezing container are pre-chilled to 2-8°C. Maintain cryopreservation medium, resuspend the cell pellet and aliquot cell suspension on ice when many vials are being frozen. Optional multi-step protocol: cryogenic vials containing cells are kept at 2-8°C for 0.5 hour, followed by -20°C for 2 hours, followed by -80°C overnight, followed by long-term storage in liquid nitrogen.

### 5. Thaw human PSCs

- (1) Dilute cryopreservation medium with ten times the volume of DMEM/F12 at room temperature to a 15 ml conical tube.
- (2) Quickly thaw the cells in a 37°C water bath by gently shaking the cryogenic vial continuously until only a small frozen cell pellet remains. Wipe the cryogenic vial with 70% isopropanol to sterilize.
- (3) Transfer the contents of the cryogenic vial to the 15 ml conical tube containing DMEM/F12. Mix gently. Centrifuge cells at 400×g for 3 minutes, then remove supernatant. Resuspend the cell pellet in pre-warmed complete *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium (15-25°C). Seed cells into culture vessels pre-coated with vitronectin.

Note: Addition of 10 μM ROCK inhibitor Y-27632 to culture medium could improve the survival and cloning efficiency of hPSCs after thawing.

### Notes

- Do not freeze and thaw *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Supplement repeatedly. Store supplement aliquots for one-time use prior to use.
- Thaw *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Supplement at 2-8°C or room temperature. Do not thaw in a 37°C water bath.
- Complete *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium should be stored at 2-8°C in the dark for up to 2 weeks. Do not re-freeze.
- Prior to use, warm Complete *TransStem*<sup>TM</sup> Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium to room temperature, not in a 37°C water bath.

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