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 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 *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 amount of 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^2 per well	1.0 ml
12-well plate	4 cm^2 per well	0.5 ml
24-well plate	2 cm^2 per well	0.25 ml
35-mm dish	10 cm^2	1.0 ml
60-mm dish	20 cm^2	2.0 ml
100-mm dish	60 cm^2	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*™ 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*™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium can be cryopreserved using *TransStem*™ 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 1-2×10^6 cells per cryogenic vial. One well of a 6-well plate gives rise to approximately 1-2×10^6 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, -20°C for 2 hours, -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*™ 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*™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Supplement repeatedly. Store supplement aliquots for one-time use prior to use.
- Thaw *TransStem*™ 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*™ 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*™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium to room temperature, not in a 37°C water bath.

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