

StemGro® TeSR Pluripotent Stem Cell Medium-3D

Cat.No.	Product	Amount	Shelf Life	Type	Storage	Transportation
T441KJ	StemGro® TeSR Pluripotent Stem Cell Medium-3D	Set				
T435KH	StemGro® ESS8/TeSR Basal Medium AOF/HOF	450 mL	12 months	Liquid	2-8 °C	Blue Ice
S933JJ	StemGro® TeSR-3D Supplement, 10X, AOF/HOF	50 mL	12 months	Liquid	-30 ~ -5 °C	Dry Ice



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BasalMedia

1.Introductions

When iPSCs are used for drug screening or cell therapy, a large-scale expansion process is required. Compared with traditional two-dimensional planar culture that relies on increasing the culture surface area, three-dimensional (3D) suspension culture represents the mainstream direction for industrialization. In this process, iPSCs are cultured in suspension as small aggregates or on microcarriers in bioreactors (such as stirred-tank bioreactors and wave-mixed bioreactors).

StemGro® TeSR Pluripotent Stem Cell Medium - 3D is a chemically defined, serum-free medium specifically designed for the growth and expansion of human pluripotent stem cells (hPSCs) and human embryonic stem cells (hESCs) under 3D suspension culture conditions. The medium consists of two components: StemGro® ESS8/TeSR Basal Medium and StemGro® TeSR-3D Supplement. The supplement contains recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor (rh TGFβ), eliminating the need to add other growth factors. Thanks to the addition of long-acting cytokines, a single medium change can sustain the culture for over 48 hours, allowing a flexible culture schedule and eliminating the need for weekend medium changes.

In addition, Yuanpei also offers the StemGro® TeSR Pluripotent Stem Cell Medium Culture Kit, specifically designed for two-dimensional planar culture of iPSCs, catalog number T440KJ.

This product is prepared using Water for Injection (WFI).

2. Quality management system

The product is manufactured in CGMP standard workshop in Basalmedia Shanghai.

Basalmedia Shanghai Biotechnology Co.Ltd has obtained ISO9001 :2015 , ISO13485 :2016 quality system certification.

3.Product Parameters

StemGro® ESS8/TeSR Basal Medium AOF/HOF (T435KH)

The product is sterilized by filtration.
Appearance: Transparent liquid with red color
Endotoxin: ≤1 EU/mL
Osmotic pressure: 340 ~ 400 mOsm/kg-H₂O
pH value: 7.0 ~ 7.4
Storage: 2 ~ 8 °C
Transportation: Blue Ice
Application: for research and production only.

StemGro® TeSR-2DSupplement, 10X, AOF/HOF (S932JJ)

The product is sterilized by filtration.
Appearance: Transparent liquid with Light Yellow color
Endotoxin: Check & Record
Osmotic pressure: Check & Record
pH value: Check & Record
Storage: -30 ~ -5 °C
Transportation: Dry Ice
Application: for research and production only.

4.User guide

Please wear appropriate safety gloves, lab clothes and goggles when using. The product should not be used on the human body.

The environment in which the cells contact directly must be sterile, and the reagents used in cells culture must be sterile. Please conduct cell experiments in a sterile environment. Before moving any vessels or tools into a sterile environment, remove

the outer packaging at the entrance and disinfect with alcohol swab.

Note: Do not use the product when it has completely melted upon receipt, or when it has passed its term of validity.

5. Preparation of StemGro® TeSR Complete Medium

StemGro® TeSR-3D Supplement is frozen prior to use. Thaw gently at 2–8 °C, mix by swirling gently, and then slowly add to StemGro® ESS8/TeSR Basal Medium with gentle swirling to ensure a homogeneous medium. **Do not thaw the supplement at 37 °C.** StemGro® TeSR Complete Medium can be stored at 2–8 °C for 2 weeks.

6. Cell Culture Conditions

StemGro® TeSR Complete Medium does not require the addition of any other components. If contamination of the cultured stem cells is a concern, antibiotics may be added prior to use. For example, gentamicin at a final concentration of 5 µg/mL.

Applicable cell lines: ESCs, iPSCs

Cell type: adherent cells

Culture vessels and equipment: culture flasks and a CO₂ incubator

Culture conditions: 36–38 °C, humidified air with 5% CO₂, protected from light.

Before conducting experiments, set the temperature and gas parameters of the cell culture equipment

7. Recover cryopreserved cells

1. Rapidly thaw a vial of frozen cells in a 37 °C water bath (< 1 minute). When the last ice crystal has just melted, immediately remove the cryovial from the water bath.
2. Gently aspirate the contents of the vial and transfer to a 50 mL sterile centrifuge tube.
3. Slowly add pre-warmed StemGro® TeSR Complete Medium while gently swirling the centrifuge tube to ensure mixing.
4. Centrifuge at 100–200 × g for 5 minutes at room temperature, then aspirate the supernatant.

5. Resuspend the cells in a minimal volume of medium, count using a cell counter, and calculate the viable cell density.
6. In a T75 culture flask that has been rinsed with medium, add an appropriate volume of pre-warmed complete StemGro® TeSR Medium, then add the cell suspension to achieve a viable cell seeding density of approximately 5×10^3 cells/cm².
7. Place the flask in the incubator for culture.
8. Replace the medium every 2–3 days. Fresh medium should be pre-warmed before use.

8. Cell Passage

Example of using a T75 culture flask as an example for passaging PSCs:

1. Passage the cells when the cell confluence reaches 85%.
2. It is recommended to use Trpzyme® iPSC/ES Cell Dissociation Solution (animal origin-free, Yuanpei catalog number S349JV) and StemGro® TeSR Complete Medium.
3. Aspirate the medium from the T75 culture flask. Rinse the cell monolayer with DPBS without calcium and magnesium, then aspirate the rinse solution.
4. Add 5–8 mL of pre-warmed recombinant trypsin solution to each T75 flask, ensuring the liquid covers the entire culture surface. Incubate under the recommended cell culture conditions for 5–10 minutes.
5. Observe the culture flask under an inverted microscope to ensure that the cells have completely detached.
6. Then add 5–10 mL of pre-warmed DPBS with calcium and magnesium to each flask, ensuring the buffer completely covers the culture surface. Transfer the cell suspension to a 15 mL sterile centrifuge tube. Rinse the flask again with 5–10 mL of DPBS with calcium and magnesium and collect the liquid into the centrifuge tube.
7. Centrifuge at 100–200 × g for 5 minutes at room temperature and carefully aspirate the supernatant.
8. Resuspend the cells in a minimal volume of StemGro® TeSR Complete Medium and perform a viable cell count.
9. Add 15 mL of pre-warmed StemGro® TeSR Complete Medium to a culture flask pre-coated with vitronectin.
10. Seed the cells at a viable cell density of 5×10^3 cells/cm²

(i.e., 3.75×10^5 viable cells per T75 flask). Gently swirl the flask to ensure even cell distribution.

11. Place the cells under the recommended culture conditions.
12. Replace the medium every 2–3 days.

9. Cryopreserve cells

Before the cryopreservation procedure, prepare a sufficient number of cells at 80–90% confluence:

1. Prepare cryopreservation medium (46.25% fresh complete medium + 46.25% conditioned medium (i.e., medium that has been used to culture the cells) + 7.5% DMSO) and pre-cool it at 2–8 °C protected from light (for no more than 24 hours); alternatively, it is recommended to use Yuanpei Biotech CD-Freezer® Chemically Defined Cell Cryopreservation Solution (a chemically defined, serum-free formulation containing 7.5% DMSO, Yuanpei catalog number S919JV).
2. Determine the cell number and calculate the required volume V of cryopreservation medium (recommended cell freezing density: $1-5 \times 10^6$ cells/mL).
3. Aspirate the medium from the culture flask, then rinse the adherent cells with 5 mL of DPBS without Ca^{2+} and Mg^{2+} . Aspirate the DPBS rinse solution after washing.
4. Add 3 mL of recombinant trypsin solution (only 1 mL is

needed for a T25 flask).

5. Let stand at room temperature for 2–5 minutes; during this time, gently tap the flask wall to help the cells detach. Once the cells have detached from the flask surface, immediately add V mL of the cryopreservation medium prepared in step 1. Tilt and swirl the flask gently to mix the newly added liquid and ensure it comes into full contact with all inner surfaces of the flask.
6. Depending on subsequent needs, dispense the cell suspension into cryovials (typically 1.5 mL per vial).
7. Label the cryovials appropriately (e.g., cell name, date of cryopreservation, and operator).
8. Use a controlled-rate freezer to manage the temperature decrease (standard freezing rate: -1 to -2 °C/min). Once the temperature reaches below -25 °C, the cooling rate can be increased to -5 to -10 °C/min; upon reaching -100 °C, the vials can be rapidly immersed in liquid nitrogen.
9. Alternatively, a manual cooling method can be used: place the cryovials into a freezing container containing isopropanol (Nalgene), put the container in a -20 °C freezer for 2 hours, then transfer to a -80 °C freezer overnight. Finally, remove the cryovials individually and transfer them into a liquid nitrogen container.

10. Related Products

Cat.No	Product	Amount	Storage	Transportation
T430KJ	StemGro® ESS8 Pluripotent Stem Cell Medium	Set		
T440KJ	StemGro® TeSR Pluripotent Stem Cell Medium	Set		
S349JV	Trpzyme® iPSC/ES Cell Dissociation Solution, Phenol Red-Free	100 mL	2 ~ 30 °C	RT
S342JV	Trpzyme® Recombinant Trypsin Solution, no phenol red	100 mL	2 ~ 8 °C	Blue Ice
S919JV	CD-Freezer® chemically defined cryopreservation medium	100 mL	2 ~ 8 °C	Blue Ice
S924JV	CD-Freezer® chemically defined cryopreservation medium HD	100 mL	2 ~ 8 °C	Blue Ice
B210KJ	Dulbecco's DPBS, without Ca^{2+} , Mg^{2+} , Phenol red	500 mL	2 ~ 30 °C	RT
B220KJ	Dulbecco's DPBS, with Ca^{2+} and Mg^{2+} , without Phenol red	500 mL	2 ~ 30 °C	RT