

StemGro® M4Diff Medium

Cat.No.	Product	Amount	Shelf Life	Type	Storage	Transportation
T460KJ	StemGro® M4Diff Medium	500 mL	12 Months	Liquid	2-8 °C	Blue Ice



1.Introductions

StemGro®M4Diff Medium is a chemically defined, serum-free, and animal component-free medium designed for inducing human embryonic stem cells (hESCs) and human pluripotent stem cells (hPSCs).

This medium can be used in both adherent and embryoid body (EB) differentiation protocols. When combined with various induction factors or cytokines, it can support differentiation along the ectoderm, mesoderm, and endoderm lineages.

This product does not contain PVA. Before use, 5 mL of PVA solution (S508J4) can be additionally added. In addition, Yuanpei can customize an insulin-free M4Diff Medium for cardiomyocyte differentiation.

Furthermore, in differentiation experiments, this medium can also be mixed with other basal media such as DMEM/F12, RPMI 1640, DMEM, TeSR/ESS6, and ESS6, typically added at a ratio of 20% of the final total volume.

Product Features:

- Compatible with ES or PSC cultures maintained in E8 and TeSR
- Compatible with both adherent and embryoid body (EB) differentiation protocols
- Supports multi-lineage differentiation into ectoderm, mesoderm, and endoderm

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

The product is sterilized by filtration.

Appearance: Transparent liquid with red color

Endotoxin: ≤1 EU/mL

Osmotic pressure: 280 ~ 320 mOsm/kg-H₂O

pH value: 7.0 ~ 7.4

Storage: 2 ~ -8 °C

Transportation: Blue Ice

Application: for research and production only.

3.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.

4.Preparation of StemGro®M4Diff Complete Differentiation Medium

Before use, appropriate induction factors or cytokines must be added to StemGro® M4Diff Medium to prepare the complete differentiation medium. The complete medium can be stored at 2-8 °C for 2 weeks.

5.Culture Conditions

Medium: M4Diff Complete Differentiation Medium

Cell lines: human embryonic stem cells (hESCs), human pluripotent stem cells (hPSCs)

Differentiation protocol: Adherent or embryoid body (EB)

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.

6.Recover cryopreserved ESC/PSC 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 dropwise StemGro®TeSR Medium that has been warmed to room temperature, while gently swirling the centrifuge tube to ensure mixing;
4. Centrifuge at $200 - 300 \times g$ for 5 minutes at room temperature, then aspirate the supernatant;
5. Resuspend the cells in an appropriate volume of complete 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 $1-2 \times 10^4$ cells/cm²;
7. Place the flask in a 37 °C, 5% CO₂ incubator and static culture it;
8. Replace the medium every 2–3 days. Fresh medium should be warmed to room temperature before use.
7. Centrifuge at $200 - 300 \times g$ for 5 minutes at room temperature and carefully aspirate the supernatant;
8. Resuspend the cells in an appropriate volume of StemGro®TeSR Complete Medium and perform a viable cell count;
9. Add 15 mL of pre-warmed StemGro®TeSR Complete Medium (warmed to room temperature) to a culture flask pre-coated with vitronectin;
10. Seed the cells at a viable cell density of $1-3 \times 10^4$ cells/cm² (i.e., $7.5-21.5 \times 10^5$ viable cells per T75 flask). Gently swirl the flask to ensure even cell distribution;
11. Add Y-27632 (S504J0) or CEPT (S928J0) at a dilution of 1:1000;
12. Place the cells in a 37 °C, 5% CO₂ incubator and static culture it;
13. After 24 hours of culture, replace the medium with StemGro®TeSR Complete Medium;
14. Replace the medium every 1–3 days.

7.ESC/PSC Cell Passage

Passaging of PSCs Using TeSR Medium in a T75 Culture Flask as an Example

1. Passage the cells when they reach 70–85% confluence in vitronectin (VTN)-coated dishes;
2. Before use, pre-warm iPSC/ES Cell Dissociation Solution (S349JV) and StemGro® TeSR Complete Medium to room temperature;
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 3–5 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 3–10 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 without 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

8.Cryopreserve ESC/PSC cells

Before starting the cryopreservation procedure, prepare a sufficient number of ESC/PSC cells at 70–85% 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, 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 Ca²⁺ and Mg²⁺. Aspirate the DPBS rinse solution after washing.
4. Add 3–5 mL of recombinant trypsin solution (only 1 mL is needed for a T25 flask).
5. Let stand at room temperature for 3–10 minutes; during this time, gently tap the flask wall to help the cells detach. Once

the cells have detached from the flask surface, add fresh complete medium to stop the digestion. Collect the cells into a 15 mL centrifuge tube, centrifuge at $200\text{--}300 \times g$ for 5 minutes at room temperature, discard the supernatant, and then immediately add V mL of the cryopreservation medium prepared in step 1. Gently tilt and swirl the centrifuge tube to mix the newly added liquid and ensure it comes into full contact with the cell pellet.

6. Depending on subsequent needs, mix the cell suspension thoroughly and dispense into cryovials as required (generally 1 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 transferred into liquid nitrogen for storage.
9. Alternatively, a manual cooling method can be used: place the cryovials into a freezing container containing isopropanol, 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.

9.Related Products

Cat.No.	Product	Amount	Storage	Transportation
T430KJ	StemGro® ESS8 Pluripotent Stem Cell Medium	Set		
T440KJ	StemGro® TeSR Pluripotent Stem Cell Medium	Set		
T463KJ	StemGro® ESS6 Medium	500 mL	$2 \sim 8$ °C	Blue Ice
T464KJ	StemGro® TeSR/ESS6 Medium	500 mL	$2 \sim 8$ °C	Blue Ice
M251R0	rhLaminin-521 1mg/mL AOF	1 mL	$-30 \sim -5$ °C	Dry Ice
M252R0	Vitronectin(VTN) 1mg/mL AOF	1 mL	$-30 \sim -5$ °C	Dry Ice
S508J4	10% PVA Solution	5 mL	$-30 \sim -5$ °C	Dry Ice
S349JV	Trpzyme® iPSC/ESC Cell Dissociation Solution, Phenol Red-Free	100 mL	$2 \sim -30$ °C	RT
B391JV	0.5 mM EDTA Solution	100 mL	$2 \sim -30$ °C	RT
S504J0	10mM Y-27632Solution (1000X) , Animal Component free	100 mL	$-30 \sim -5$ °C	Dry Ice
S928J0	CEPT Supplement, 1000X	1 mL	$-30 \sim -5$ °C	Dry Ice
S919JV	CD-Freezer® chemically defined cryopreservation medium	100 mL	$2 \sim 8$ °C	Blue Ice
B210KJ	Dulbecco's DPBS, without Ca^{2+} , Mg^{2+} , Phenol red	500 mL	$2 \sim 30$ °C	RT