hiPSCs/hESCs serum-free and animal component-free (ACF) medium Instruction

【Product Identification】

hiPSCs/hESCs serum-free and animal component-free (ACF) medium

【Catalog Number】MCM-005

【Packaging Specifications】

Basal culture medium (400 mL/bottle)

5×Supplement (100mL/bottle)

【Intended Use】

This product is suitable for the maintenance and expansion culture of human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs). It can disperse large, undifferentiated stem cell colonies into smaller colonies or single cells, allowing them to detach from the culture surface. Cells dissociated with this product exhibit high viability and maintain a good growth state after passaging. Please note that this product does not possess the ability to induce or differentiate the stem cells.

【Storage Conditions & Shelf Life】

Basal culture medium：

Storage: Protect from light at 2-8°C.

Shelf Life: 6 months.

5×Supplement：

Storage: Protect from light at -25 ~ -15℃.

Shelf Life: 6 months.

【Sample Requirements】

The pluripotent stem cells cultured using this medium are human iPSCs or ESCs, maintained in a sterile environment, and have not undergone differentiation.

【Protocol】

1. Preparation

Allow the basal medium and the 5× Supplement to room temperature (Do not place them in the incubator or water bath). It is recommended to place the 5× Supplement in a refrigerator at 2-8°C two days in advance for thawing. Once the 5× Supplement is completely thawed, aliquot it into appropriate volumes (ensure it is thoroughly mixed before aliquoting).

2. Preparation of complete medium

Mix the basal medium and the 5× Supplement uniformly in a 4:1 volume ratio to prepare the complete medium. The complete

medium can be stored for up to 2 weeks under dark conditions at 2 ~ 8°C. Before use, ensure the complete medium is brought back to room temperature.

3. Resuscitation of hiPSCs/hESCs

Thaw hiPSCs or hESCs. Coat the plate wells or cell culture flasks with coating proteins such as Laminin-521 or Vitronectin. Add 10 μM Y-27632 to the complete medium, then resuspend the cells and seed them onto the plate or flask (refer to the table below for the resuspension volume). Place them in a 37°C, 5% CO2 incubator for culture. On the second day, replace the medium with a complete medium without Y-27632.

Cell culture system	12-well plate	6-well plate	T25 culture flask
Recommended dosage	1mL	2mL	5mL
If the culture system you are using is not listed in the table above, please calculate based on the bottom area of the cell culture container.			

4. Maintenance of hiPSCs or hESCs

Change the complete medium completely every 2 days (refer to the table above for the volume of the complete medium). Before changing the medium, observe the culture status of the stem cell colonies, and promptly remove any colonies that have already differentiated. After replacing the medium with an equal volume of fresh complete medium, return the cell culture container to the incubator to continue the culture. It should be avoided to expose the stem cell colonies to air for extended periods; some residual liquid can be retained to keep the stem cell colonies moist.

If stem cell colonies grow too large (diameter exceeding 1mm), if the edges of the colonies begin to touch, or if the centers of the colonies start to show signs of thickening and aging, the cells need to be passaged. Under normal conditions, passaging is required approximately every 5-6 days. Before passaging, remove any already differentiated stem cell colonies. Fragment the undifferentiated stem cell colonies using either the cutting method or the dissociation method* (for the dissociation method, it is recommended to use the company's stem cell enzyme-free dissociation solution Disperser (Catalog No.: MCL-014)). Resuspend the stem cell colony fragments in a complete medium (refer to the table above for the resuspension volume), and seed the fragments onto the wells or flasks coated with coating protein. Gently swirl the cell culture container in a cross pattern to distribute the colony fragments evenly. Place the culture container into a 37°C, 5% CO_2 incubator for culture.

*Note: If using dissociation reagents or enzymes such as TryPLE, EDTA, or GCDR to digest and dissociate the stem cell colonies, it is strongly recommended to add 10μM Y-27632 to the resuspending complete medium as an anti-apoptotic agent. The medium should be changed to Y-27632-free complete medium on the second day after passaging.

6. Cryopreservation of hiPSCs/hESCs

It is recommended to use CryoStor® CS10 Freezing Media (Biolife Solutions) and other cryopreservation media to cryopreserve

hiPSCs/hESCs. After the hiPSCs/hESCs clones in good culture status are fragmented by cutting or dissociation method, they are resuspended in cryopreservation media, dispensed into cryopreservation tubes, labeled, and cryopreserved according to the requirements of the cryopreservation media manufacturer.

【Product Performance Specifications】

1. Appearance: The label needs to be clear and easily identifiable. There should be no leakage of the liquid. The basic culture medium is a red-prepared liquid, and the 5× Supplement is a pink-prepared liquid.

2. Clarity: The liquid should be clear and free from any visible particles or impurities.

3. pH：7.1

4. Osmolality：290-330mOsm/kg

5. Bacterial Endotoxins：<0.5EU/mL

6. Sterility Testing: No microbial growth detected.

7. Mycoplasma Testing: Negative for mycoplasma contamination.

【Precautions】

1. After opening, store at 2~8°C.

2. The entire operation process should be carried out under sterile conditions to avoid microbial contamination.

3. If contact with the skin occurs during use, wash immediately with water. If discomfort persists, seek medical attention promptly.

4. Do not use it if the packaging shows cracks or damage.

5. Expired products must not be used.

【Manufacturer Information】

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