Serum-Free Cell Freezing Medium

Cat#：6032011

[Product Name]
Serum-Free Cell Freezing Medium

[Model Specification]

Product Name	Cat. No.	Size	Quantity
Serum-Free Cell Freezing Medium	6032011	100 mL	1 bottle

[Product Description]
Serum-Free Cell Freezing Medium is a chemically defined freezing medium developed by Dakewe on the basis of lymphocyte freezing kit, which has clear components and contains neither xenogeneic animal-derived components nor serum. On the basis of obtaining high cell viability and cell yield, this product can effectively avoid the change of serum quality and the impact of serum components and exogenous components on experimental research, and achieve excellent cell freezing and thawing effects.

[Direction for Use]
Reagents and instruments requiring self-preparation by the experimenter:
- Cell medium (commonly used for culturing cells requiring freezing);
- Clean bench;
- Temperature-controlled centrifuge, equipped with horizontal rotor with 15 mL and 50 mL centrifuge tubes, and recommended use of professional cell centrifuge; the sterile conical tube should be used as the centrifuge tube;
- P20, P200 and P1000 micropipettes and pipette tips, pipette aids and sterile serological pipettes;
- Sterile 0.5 mL and 1.5 mL EP tubes;
- Cell counter;
- Freezing container and sterile cryotube;
- -80℃ freezer;
- Liquid nitrogen tank;
- Water bath at 37°C.

Cell Freezing
1. Mark the cryotubes to be used in detail. Place the cryotubes at -20℃ for thermal equilibration, and place the freezing container, cell medium and serum-free cell freezing medium at 4℃ for thermal equilibration.
2. Count the cells to be frozen, and centrifuge 250 g at 4°C for 10 min.
3. (Operation on ice) Discard the supernatant and resuspend with the cell medium equilibrated at 4℃ according to the results of cell count, so that the cell concentration is adjusted to twice the freezing concentration (e.g., if the cell concentration to be frozen is 1×10^7 cells/mL, adjust the cell concentration to 2×10^7 cells/mL with the medium), and then blow the cells off with the pipette evenly.

Notes: The density of freezing cells recommended for this product is $5 \times 10^5 \sim 2 \times 10^7$ cells/mL, and the appropriate density of freezing cells should be selected according to the nature of freezing cells and subsequent experimental arrangements during freezing.

4. (Operation on ice) Slowly add an equal volume of serum-free cell freezing medium after placement in an ice bath dropwise, and mix gently.

Notes: Be sure to add dropwise to avoid rapid changes in DMSO concentration in the medium.

5. (Operation on ice) Dispense 1 mL of cell suspension per tube into freezing tubes equilibrated at -20°C, and then screw the cap tightly on the freezing tubes.

6. Immediately place the freezing tube in the freezing container that has been equilibrated at 4℃, and then place the freezing container in a -80℃ freezer. Freezing tubes can also be placed in a programmable cooler to cool to -80℃ at a rate of 1℃/min.
7. Transfer freezing tubes stored at -80°C to liquid nitrogen 24 h later.
8. Record the cell freezing location in the liquid nitrogen tank on the liquid nitrogen equipment management sheet in the laboratory.

Cell Thawing

1. Perform thermal equilibration on cell medium at 4°C. Perform thermal equilibration on 50 mL and 15 mL centrifuge tubes at 4°C. Preadjust the water bath to 37°C.
2. Remove the cryotubes from liquid nitrogen with forceps. If liquid nitrogen leaks into the freezing tube, unscrew the cap slightly to allow the vaporized liquid nitrogen to escape.

Warning: Some freezing tubes have been reported to explode upon thawing, and to eliminate this hazard, it is recommended that only firm polyethylene freezing tubes be used for liquid nitrogen preservation in experiments.

3. Clamp the freezing tube with forceps and immerse it in the water bath at 37℃ (note that the pipette tip should not be immersed below the liquid level). Gently shake the freezing tube and pay attention to the thawing of freezing cells in the tube. When the ice nucleus becomes soybean-sized in the tube, take it out, wipe the tube body with alcohol cotton, and transfer it to a clean bench.

Notes: This step is critical for cell viability. It is necessary to thaw as soon as possible to reduce the damage to the cells during thawing; in addition, the time of cells staying at high temperature should be reduced as much as possible to reduce the toxicity of DMSO to cells. Do not leave the freezing tube in the water bath.

4. (Operation on ice) Open the cap of the freezing tube. Gently pipette the cell suspension (approximately 1 mL) from the freezing tube into a precooled 15 mL centrifuge tube. Slowly add 5 mL of cell medium (4℃), gently shake while adding, cover the tube cap upon completion of dropping, and mix well by gentle inversion.
5. Continue to add fill up with the medium slowly (4℃) to 14 mL, screw the cap, and mix by gentle inversion.
6. Centrifuge 250 g for 10 min at 4°C. Carefully aspirate the supernatant, and the supernatant is required to be aspirated as far as possible without touching the cells pelleted in the tube bottom. Disperse the cell mass by flicking the finger in the bottom of the centrifuge tube. Add 1 mL of medium and resuspend cells.

Notes: Aspirating the supernatant is more effective than decanting the supernatant, contributing to reduce the residual DMSO; finger flicking of the centrifuge tube can gently disperse the cell mass. Do not blow and aspire with a gun, otherwise it is harmful to the cells.

7. (Optimization step, can be omitted) Cell clumping is caused by entanglement of DNA released from dead cells. 0.1 mL of DNase (1 mg/mL) can be added and incubated at 37°C for 10 min.
8. Take 50 μL of cell suspension and count trypan blue viable cells to calculate viability. Description: Viability = number of viable cells/total number of cells × 100%. In the presence of trypan blue, viable cells are colorless and translucent, while dead cells are blue and dull.
9. According to the counting results, fill up with cell medium to the desired cell concentration for subsequent assay.

[Description of Product Symbol]

Product Symbol	Description	Product Symbol	Description
REF	Catalogue number	LOT	Batch code
	Date of manufacture		Temperature limit
	Use-by date		Consult instructions for use

Product Symbol	Description	Product Symbol	Description
	Manufacturer		Keep away from sunlight

[Preservation Method]
Keep away from light at 2℃~8℃ , valid for 12 months.

[Precautions]
Avoid repeated freezing and thawing of this product, and pay attention to operate aseptically during use.

[Related Products]

Product Name	Cat No.	Size
DPBS	6062011	500 mL/bottle
Trypsin Solution	6063111	100 mL/bottle
GF20 Plus MSC Xeno-Free SFM	6914132	500 mL/kit
	6914142	500 mL/kit
GF20 MSC Xeno-Free SFM	6914112	500 mL/kit
	6914122	500 mL/kit
293 Growth Serum-Free Medium	6117111	1000 mL/bottle
CHO Growth Serum-Free Medium	6217011	1000 mL/bottle
L100 Serum-Free Medium for Lymphocyte	6911011	1000 mL/bottle
L500 Serum-Free Medium for Lymphocyte	6111021	1000 mL/bottle
L500 Serum-Free Medium for Lymphocyte (GMP)	6811021	1000 mL/bottle
Lymphocyte Separation Tube for Human Peripheral Blood	7922112	15 mL × 20 vials
	7922021	50 mL × 25 vials
Density Reagent	7912011	250 mL/bottle
Human Lymphocyte Separation Medium	7111011	100 mL/bottle
Cell Culture Supplemental Mix	6122012	25 mL
	6122011	250 mL

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[Company Information]
Manufacturer and after-sales service unit Name:
Shenzhen Dakewe Bio-engineering Co., Ltd.
Website:www.dakewe.com
Telephone: (86-755) 86235300
Email: RD@dakewe.com
Address: Room 702-703, Building No.1, Shenzhen Biomedicine Innovations Industrial Park,No.14 Jinhui Road, Kengzi Street, Pingshan District, Shenzhen, China
After-sales service telephone: (86-755) 86235300
Zip Code: 518122