





## SuperKine™ Serum/Protein-Free Cell Freezing Medium

Cat #: BMU108-EN

Size: 100 mL

	<b>Serum/Protein-Free Cell Freezing Medium</b>		
	<b>Cat #:</b> BMU108-EN		<b>Lot #:</b> Refer to product label
	<b>Storage:</b> Stored at 4°C for 12 months, and stored at -20°C for a long time.		

### Assay Principle

SuperKine™ Serum/Protein-Free Cell Freezing Medium is a kind of non programmed, ready-to-use cell freezing medium with clear formula, serum-free and protein-free, which is applicable to most mammalian cells. It can eliminate the potential pathogen contamination or immune reaction risk of serum or protein, so it is safer to use, no need for programmed cooling, and can keep the recovery survival rate of various cells above 95%.

### Materials Supplied and Storage Conditions

Components	Size	Storage conditions
Serum/Protein-Free Cell Freezing Medium	100 mL	4°C

### Assay Procedure

#### I Cell Freezing

1. For adherent cells:

(1) Remove supernatant of the culture medium, wash cells with sterile PBS for 1-2 times, then an appropriate amount of 0.25% trypsin to cover the cells at the bottom of the whole cell culture flask or dish, cover it and put it into the cell incubator for digestion (The digestion time should be judged according to the cell morphology under the microscope). Observe the cells under the microscope, when the cells were round and about to break off the wall the wall but not break off the wall (It is recommended to use a pipette to gently blow to confirm whether the cells are free of the wall), add complete culture medium to terminate the digestion, and then blow the cells down gently.

(2) Transfer the cell suspension to 15 mL sterile centrifuge tube, counting cells.

(3) Centrifuge at 1,000 rpm for 5 min.

**Note: The speed and time of centrifugation depends on the cell type.**

(4) Discard the supernatant of the culture medium and add the Cell Freezing Medium to resuspend the cells, the addition amount of Cell Freezing Medium is according to the final density of  $1-10 \times 10^6$  cells/mL.

(5) The cell suspension was separated into cell freezing tubes and marked.

(6) The separated cell freezing tubes were directly placed in the -80°C refrigerator.

(7) If liquid nitrogen is needed, it should be stored in -80°C refrigerator for 24 h and then moved to the liquid nitrogen tank.

2. For non-adherent cells:

(1) Transfer the cell suspension to 15 mL sterile centrifuge tube, counting cells.

(2) Centrifuge at 1,000 rpm for 5 min.

**Note: The speed and time of centrifugation depends on the cell type.**

- (3) Discard the supernatant of the culture medium and add the Cell Freezing Medium to resuspend the cells, the addition amount of Cell Freezing Medium is according to the final density of  $1-10 \times 10^6$  cells/mL.
- (4) The cell suspension was separated into cell freezing tubes and marked.
- (5) The separated cell freezing tubes were directly placed in the  $-80^{\circ}\text{C}$  refrigerator.
- (6) If liquid nitrogen is needed, it should be stored in  $-80^{\circ}\text{C}$  refrigerator for 24 h and then moved to the liquid nitrogen tank.

## II Cell recovery

1. The cell freezing tubes were rapidly thawed in  $37^{\circ}\text{C}$  water bath.
2. Transfer the cell suspension to a sterile 15 mL centrifuge tube and add 4-5 mL complete culture medium.
3. Centrifuge at 1,000 rpm for 5 min.

**Note: The speed and time of centrifugation depends on the cell type.**

4. Remove supernatant of the culture medium, add an appropriate amount of complete culture medium and gently blow to resuspended cells, then transfer the cell suspension to the cell culture vessel, and culture in the cell incubator.

## Typical Data

The cells have been validated so far: HEK293, HeLa, CHO, MCF-7, HepG2, COS-7, RAW264.7, L929, HUVEC, SW-480, A549, hMSC, Hep3B, SK-OV-3, AE-1, MDCK, BEAS-2B, Vero, NIH-3T3, A431, PC-12, Chang liver, Jurkat, A-375, A2058, C2C12, DU-145, 769-P, HK-2, U-87 MG, U251, Raji, Siha, Saos-2.

## Precaution

1. The Cell Freezing Medium contains DMSO. For DMSO-sensitive cells, it is recommended to do a pre-experiment of freezing.
2. When freezing stem cells or primary cells, it is recommended to use this product to do a pre-experiment of freezing for at least one week, and then conduct formal freezing after confirming the performance.
3. For some special or particularly precious new cell types without seed preservation, it is recommended to use the conventional freezing medium containing serum at the same time. Only after confirming the recovery survival rate, this product can be used to ensure that the cells will not all die unexpectedly during freezing.
4. After the cells are separated, they should be moved to  $-80^{\circ}\text{C}$  refrigerator as soon as possible. If they need to be frozen for a long time, they should be transferred to liquid nitrogen.
5. It is necessary to select cells in logarithmic growth period for freezing to ensure that the cells grow well before freezing, and the proportion of live cells during cryopreservation should generally be greater than 90%.
6. For some sensitive cells, such as RAW 264.7 cells, resuscitation should be performed in time, and the corresponding medium should be centrifuged after rapid melting at  $37^{\circ}\text{C}$  to avoid differentiation.

## FAQ

1. What are the sample types applicable to this product?

It is applicable to most mammalian cells, including adherent cells and suspension cells, and 34 mammalian cell types have been validated so far.

2. How long can the cells stored in  $-80^{\circ}\text{C}$  refrigerator and liquid nitrogen be stored respectively?

The preservation time of different cells was slightly different. After testing, the cells were stored at  $-80^{\circ}\text{C}$  for more than half a year and in liquid nitrogen for more than one year.

## Recommended Products

Catalog No.	Product Name	Recommended Reason
BMU106-EN	SuperKine™ Maximum Sensitivity Cell Counting Kit-8 (CCK-8)	Detect cell proliferation
KTA1001	Live and Dead Cell Double Staining Kit	Detect cell viability

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.