EZ-cryo™ Cell Freezing Medium Product Instruction

Product Introduction

EZ-cryo™ Cell Freezing Medium is a serum-free, non-programmed cell cryopreservation solution product developed by Ubigene expert team after continuous exploration and optimization, aims to provide a more safe, efficient and convenient tool for cell cryopreservation to more scientific researchers, and to achieve a full range of protection of cells for scientific research use.

EZ-cryo™ Cell Freezing Medium has two unique protection components that can simultaneously protect cells from both intracellular and extracellular from the lethal damage caused to cells by ice crystals during the flash freezing process, and effectively improve the recovery and viability rate of the cells upon thawing. The formulation does not contain any serum of animal origin or other expensive as well as complicated components, which can effectively reduce the possible risk of contamination from animal sources and reduce the cost of cell culture. Its components are well defined and stable, with up to 90% viability upon thawing of cryopreserved cells, normal cell morphology, and significant cryopreservation performance.

Compared with the regular cryopreservation solution, this product does not require complicated programmed cooling operations, can be directly stored at -80℃ when used, followed by transferring to liquid nitrogen for storage on the next day to complete the whole cryopreservation process, saving a lot of time and effort for scientific researchers.

Product Info

Product	EZ-cryo™ Cell Freezing Medium		
Catalog 1	YK-CR-50	Quantity 1	50ml
Catalog 2	YK-CR-100	Quantity 2	100ml

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Product Validation

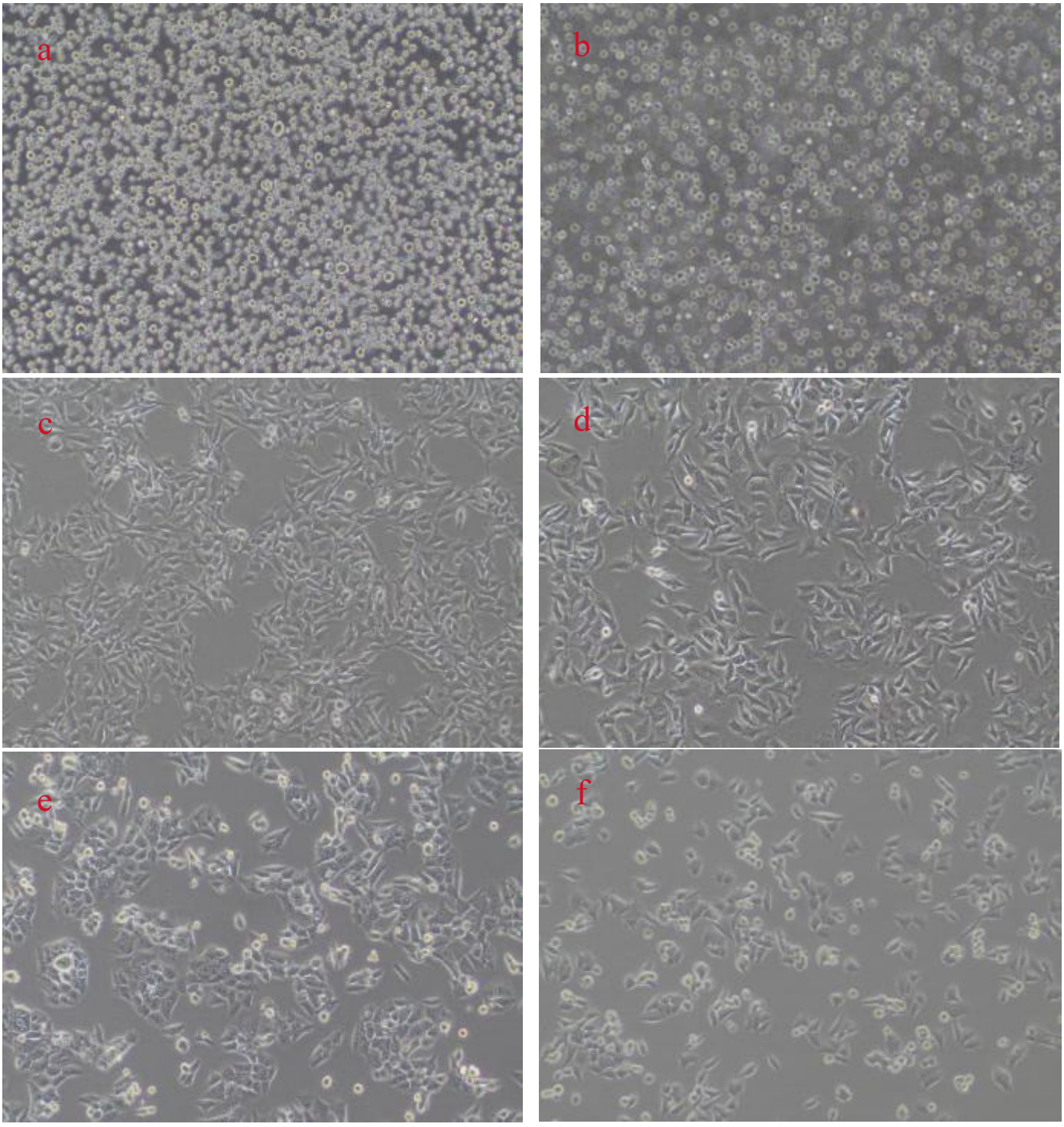

Figure 1. a and b show the cell conditions of THP-1 cryopreserved with EZ-cryo™ Cell Freezing Medium versus regular freezing medium (containing 40% serum) under 100X microscopy after 24 hours of thawing; c and d show the cell conditions of B16F10 cryopreserved with EZ-cryo™ Cell Freezing Medium versus regular freezing medium (containing 40% serum) under 100X microscopy after 24 hours

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of thawing; e and f show the cell conditions of HCT 116 cryopreserved with EZ-cryo™ Cell Freezing Medium versus regular freezing medium (containing 40% serum) under 100X microscopy after 24 hours of thawing. The validation results show that the cells cryopreserved with EZ-cryo™ Cell Freezing Medium have a higher recovery rate and better cell adhesion situation.

Product Features

- Ready-to-use, no need to dilute before use, fast and convenient for cell cryopreservation
- Serum-free, protein-free, low risk for contamination
- High viability rate upon thawing, suitable to cryopreserve the vast majority of mammalian cells
- No programmed cooling operation required, can be directly stored at -80℃ when used, fast and easy

Storage and Handling

1) Store at 4℃ and avoid direct exposure to sunlight. Shelf life: 1 year.
2) The product has stable performance and a slightly thickened appearance. Before use, it needs to mix well by pipetting

Quality Control

EZ-cryo™ Cell Freezing Medium has passed QC tests in terms of pH, osmotic pressure, bacteria, fungi, mycoplasma, and endotoxin, etc.

Cell Cryopreservation

1) Same as procedures of cell passaging, inside the ultra-clean bench, digest the cells to a single-cell suspension, and terminate digestion by adding complete medium. All liquid is transferred to a 50 ml centrifuge tube;
2) Mix well by pipetting and take 20 μL for cell counting;
3) Centrifuge at 1100 rpm for 4 mins at room temp. After centrifugation, remove and discard the supernatant, and resuspend the cells with 1-2 ml of 4℃ pre-cooled cryopreservation medium (use

the one you usually use in lab, or any commercial cryopreservation solutions are fine), then add cryopreservation medium to adjust to the required density ($1x10^6$ -$1x10^7$ cells/ml);

4) Aliquot the cell suspension to cryovials as 1 ml/tube, close the lid tightly, and the cryovials should be labeled with the cell name, source, cell passage number, and date of cryopreservation in advance;

5) Place the cryovials in -80℃ freezers;

6) Stay overnight, transfer the cryovials to liquid nitrogen for long-term storage.

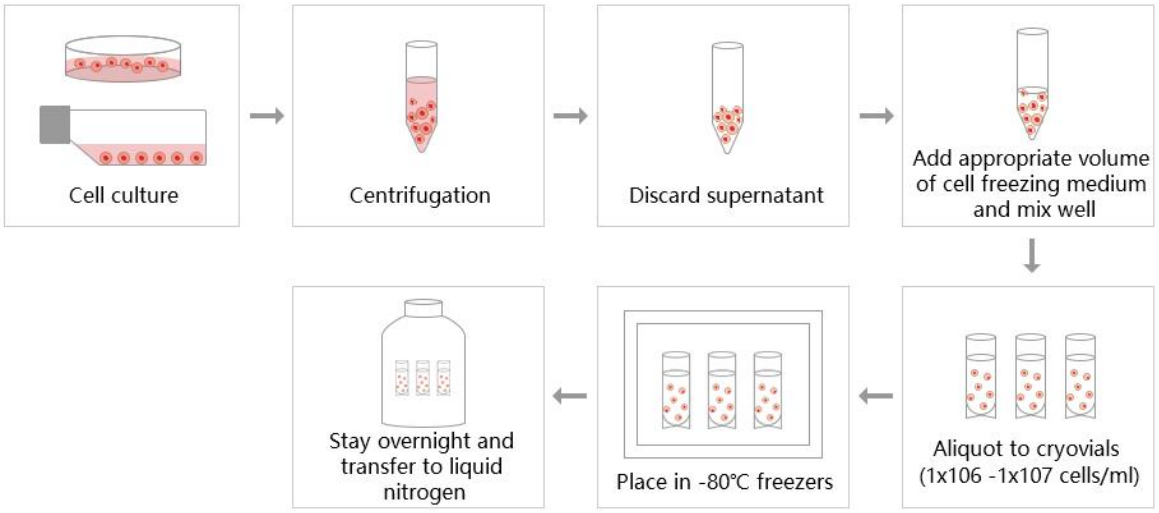

Figure 2. Workflow of cell cryopreservation

Cell Thawing

1) Preparation: warm up the complete culture medium in 37°C water bath for 30 mins. Transfer the cryopreserved vial from liquid nitrogen to - 80°C freezer, and leave for several minutes to volatilize residual liquid nitrogen;

2) Inside the ultra-clean bench, pipet 6-7 ml of complete medium into a 15 ml centrifuge tube;

3) Take out the cryopreserved vial from - 80℃ freezer and leave in dry ice temporarily, shake slightly before thawing to remove residual dry ice and liquid nitrogen. Then hold the cap with forceps, quickly thaw cells in a 37°C water bath by gently swirling the vial (Note: keep the cap out of the water). In about 1 minute, it would completely thaw;

4) Inside the ultra-clean bench, sterilize the outer surface of the vial by wiping with an alcohol cotton pellet and leave it to dry. Transfer the thawed cells to the prepared centrifuge tube (step 2) by pipette, close the lid, and centrifuge at 1100 rpm for 4 mins at room temp to collect the cells;

5) Inside the ultra-clean bench, carefully remove and discard the supernatant. Resuspend cell pellet with 1ml of fresh complete medium and then transfer to a T25 flask containing 4 ml of complete medium, label the flask with cell name, date and passage no., incubate the flask in a 37℃, 5%CO2 incubator.

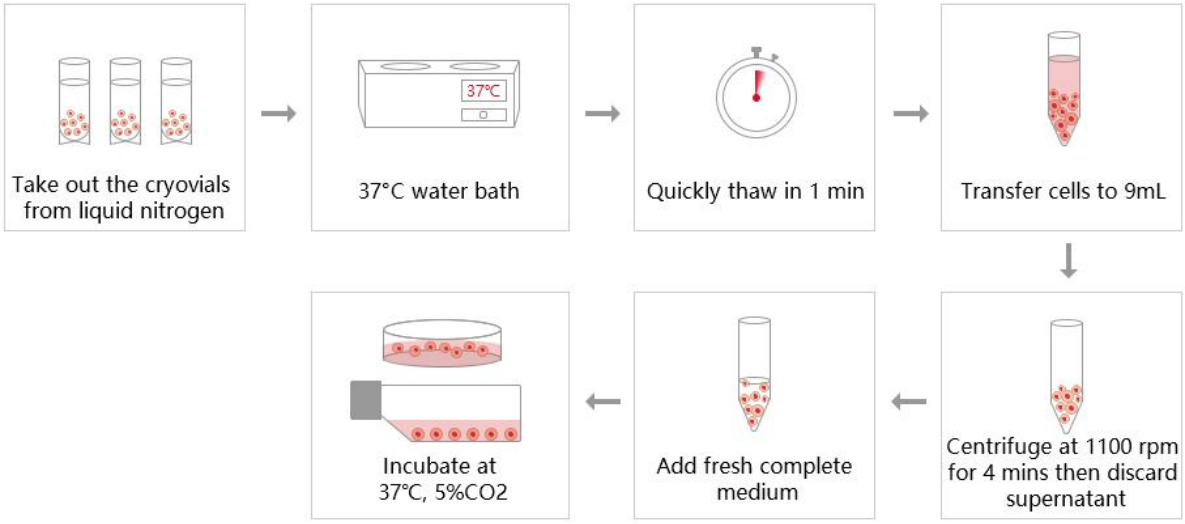

Figure 3. Workflow of cell thawing

Special Note

1. This Cell Freezing Medium contains a small amount of DMSO. After aliquoting to cryo-vials, please put the vials to - 80℃ freezer as soon as possible, avoiding placing outside for a long time. If it is temporarily inconvenient, the cells can be temporarily stored at -20℃ for a short period of time and should be transferred as soon as possible, avoiding the toxic effects of DMSO to cells.
2. This product is for scientific research purposes only.