# Product Information

FreezeMe Two, Cyropreservation Medium, Serum-free
Cat. No. FM2-F (50 ml)

## General Information

FreezeMe Two is a serum-free cryoprotective medium based on an organic preservation medium containing 10 % DMSO. Its special formulation ensures high viability of cells, making it particularly suitable for applications in stem cell research, such as embryonic and adult stem cells, induced pluripotent cells (IPS) as well as mesenchymal and hematopoietic stem cells.

Applications:

- Cryopreservation of a wide range of cell types with high viability
- Ready-to-use solution
- For cell banking
- For stem cell storage

## Product Specifications

| | |
|---|---|
| Appearance | Clear liquid |
| Storage and shelf life | Store at +2°C to +8°C. FreezeMe Two is a light sensitive solution. It should be protected from light during shipping and storage. |
| Shipping conditions | Ambient |

## Instructions for Use

Before cryopreservation cells should be checked for contamination. FreezeMe Two can be used with any standard freezing protocol.

### Cryopreservation of Suspension Cultures

Count the number of viable cells to be cryopreserved. Cells should be in mid-log phase of growth. Centrifuge the cells for 5 min to pellet cells (200 to 400 g). Remove the supernatant down to the smallest volume without disturbing the cells.
Resuspend cells in pre-cooled (+4°C to +8°C) FreezeMe Two to a concentration of $5x10^6$ to $10^7$ cells/ml.
Aliquot into cryogenic storage vials. Place vials at +4°C and start the freezing procedure within 5 min. Cells are frozen slowly at +1°C/min (by programmable coolers or by placing vials in an insulated box in a -70°C to -90°C freezer).
Then transfer storage vials to liquid nitrogen storage.

### Cryopreservation of Adherent Cultures

Detach cells from the substrate with a gentle dissociating agent. Especially with sensitive cells use Accutase (Cat. No. ACC-1B) to avoid cell damage. Inactivate dissociating agent if necessary.
Resuspend the detached cells in complete growth medium and establish the viable cell count.
Centrifuge for 5 min to pellet cells (200 to 400 g). Remove the supernatant down to the smallest volume without disturbing the cells.
Resuspend cells in pre-cooled (+4°C to +8°C) FreezeMe Two to a concentration of $5x10^6$ to $10^7$ cells/ml.
Aliquot into cryogenic storage vials. Place vials at +4°C and start the freezing procedure within 5 min. Cells are frozen slowly at +1°C/min (by programmable coolers or by placing vials in an insulated box in a -70°C to -90°C freezer).
Then transfer storage vials to liquid nitrogen storage.

# Product Information

FreezeMe Two, Cyropreservation Medium, Serum-free
Cat. No. FM2-F (50 ml)

### Thawing of Cryopreserved Cells

Cryopreserved cells can be thawed by the following procedures:

Centrifugation:

Remove cells from storage and thaw quickly in a +37°C water bath. Capricorn Scientific recommends eye protection by using approved safety goggles. We suggest the use of safety gloves to protect uncovered skin.
Place 1 to 2 ml of thawed cells in ~25 ml of complete growth medium. Mix cell suspension gently.
Centrifuge the cells at ~80 g for 2 to 3 min.
Check clarity of the supernatant and visibility of a consolidated cell pellet. Discard supernatant without disturbing the cells.
Gently resuspend the cells in complete growth medium and perform a viable cell count.
Plate the cells. Cell inoculum should be at least $3x10^5$ viable cells/ml.

Direct plating:

Remove cells from storage and thaw quickly in a +37°C water bath. Capricorn Scientific recommends eye protection by using approved safety goggles. We also suggest the use of safety gloves to protect uncovered skin.
Plate cells directly with complete growth medium. Use 10 to 20 ml of complete medium per 1 ml of frozen cells. Cell inoculum should be at least $3x10^5$ cells/ml.
Culture cells for 12 to 24 h. Replace medium with fresh complete growth medium to remove cryopreservative.

We recommend thawing procedure 1, especially when handling sensitive cells.

## Precautions and Disclaimer

This product is for research use and further manufacturing only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).