

## Neo Freeze 1, Cryopreservation Medium with Fetal Bovine Serum

#Cat: NB-58-0065 Size: 50ml

### General Information

Neo FreezeMe 1 is a cryoprotective medium that contains 10 % DMSO and 20 % FBS. The serum used is extensively tested to protect cells during cell preservation.

#### Features:

- Cryopreservation of a wide range of cell types with high viability
- Ready-to-use solution

### Product Specifications

<b>Appearance</b>	Clear red solution
<b>Storage and shelf life</b>	Store at $\leq -15^{\circ}\text{C}$ . Neo FreezeMe 1 is a light sensitive solution. It should be protected from light during storage.
<b>Shipping conditions.</b>	Frozen (Dry Ice)
<b>Thawing</b>	+37°C water bath or overnight at +2°C to +8°C. Swirl gently to homogenize.

### Quality Control

#### Freezing Protocols:

Before cryopreservation cells should be checked for contamination. Neo FreezeMe 1 can be used with any standard freezing protocol.

Cryopreservation of Suspension Cultures:

1. 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.
2. Resuspend cells in pre-cooled (+4°C to +8°C) Neo FreezeMe 1 to a concentration of  $5 \times 10^6$  to  $1 \times 10^7$  cells/ml.
3. 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).
4. Then transfer storage vials to liquid nitrogen storage.

#### Cryopreservation of Adherent Cultures:

1. Detach cells from the substrate with a gentle dissociating agent. Especially with sensitive cells use Neo Accutase Cell Detachment Solution \* (Cat.No. NB-58-0037B) to avoid cell damage. Inactivate dissociating agent if necessary.
2. Resuspend the detached cells in complete growth medium and establish the viable cell count.
3. Centrifuge for 5 min to pellet cells (200 to 400 g). Remove the supernatant down to the smallest volume without disturbing the cells.
4. Resuspend cells in pre-cooled (+4°C to +8°C) Neo FreezeMe 1 to a concentration of  $5 \times 10^6$  to  $10^7$  cells/ml.
5. 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).

6. Then transfer storage vials to liquid nitrogen storage.

### **Thawing of Cryopreserved Cells:**

Cryopreserved cells can be thawed by the following procedures:

#### **Centrifugation:**

1. Remove cells from storage and thaw quickly in a +37°C water bath. Neo Biotech recommends eye protection by using approved safety goggles. We also suggest the use of safety gloves to protect uncovered skin.
2. Place 1 to 2 ml of thawed cells in ~25 ml of complete growth medium. Mix cell suspension gently.
3. Centrifuge the cells at ~80 g for 2 to 3 min.
4. Check clarity of the supernatant and visibility of a consolidated cell pellet. Discard supernatant without disturbing the cells.
5. Gently resuspend the cells in complete growth medium and perform a viable cell count.
6. Plate the cells. Cell inoculum should be at least  $3 \times 10^5$  viable cells/ml.

#### **Direct Plating:**

1. Remove cells from storage and thaw quickly in a +37°C water bath. Neo Biotech recommends eye protection by using approved safety goggles. We also suggest the use of safety gloves to protect uncovered skin.
2. 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  $3 \times 10^5$  cells/ml.
3. Culture cells for 12 to 24 h. Replace medium with fresh complete growth medium to remove cryopreservative residues.

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

### **Precautions and Disclaimer**

This product is for research use only.

Caution: Neo FreezeMe 1 contains Dimethyl Sulfoxide (DMSO). Do not breathe gas/fumes/vapour/spray. Avoid contact with eyes and skin. Irritant to eyes, respiratory system and skin. S23 S24/25.

### **Help Needed?**

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email ([info@neo-biotech.com](mailto:info@neo-biotech.com)) or phone (+33 9 77 40 09 09).