



Cryopreservation Medium

Cat# FM-1-100

INSTRUCTION MANUAL ZBM0040.00

STORAGE CONDITIONS

Cryopreservation Medium -20°C until ready to use

Thawed medium is stable for 45 days at 4°C.

All orders are delivered via Federal Express Priority courier on dry ice.

All orders must be processed immediately upon arrival.

For *in vitro* Use Only

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

- **ZenBio, Inc.**
- **3200 Chapel Hill-Nelson Blvd., Suite 104**
- **PO Box 13888**
- **Research Triangle Park, NC 27709**
- **Telephone** (919) 547-0692
- **Facsimile (FAX)** (919) 547-0693
- **Toll Free** 1-866-ADIPOSE (866)-234-7673
- **Electronic mail (e-mail)** information@zenbio.com
- **World Wide Web** <http://www.zenbio.com>

INTRODUCTION

Zen-Bio Cryopreservation Medium (cat# FM-1-100) is suitable for the freezing of mammalian cells with average post-thaw viability of 98%. It is a serum-based cryopreservation medium optimized for the long-term storage of adipose derived adult stem cells, human preadipocytes and other primary mammalian cells in liquid nitrogen.

ITEMS INCLUDED IN THE KIT

ITEM	DESCRIPTION	UNIT	QTY	STORAGE
Cryopreservation Medium Cat# FM-1-100	100 ml serum based freeze medium for mammalian cells.	BOTTLE	1	-20°C

Other equipment/reagents required but not provided with the kit:

- Trypsin/EDTA for harvesting cells (TRP-100)
- Preadipocyte medium for neutralizing trypsin (PM-1)
- PBS for washing cells (DPBS-1000)
- Tubes for collecting cell pellets

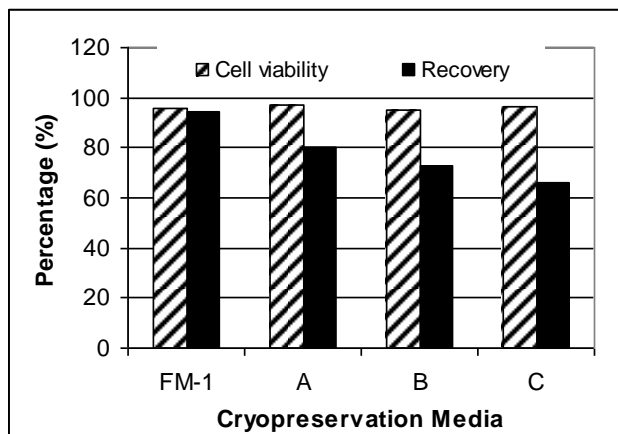
CRYOPRESERVATION PROCEDURE

1. Aspirate medium and wash cells 4-5 times using sterile Phosphate Buffered Saline (PBS) to remove all traces of serum (until there is no foaming of the medium).
2. Remove the PBS and release the cells from the cultureware bottom by adding 2 ml/T-75 flask of 0.25% trypsin/ 2.21 mM EDTA solution.
3. Incubate cells with trypsin solution for 5 minutes at 37°C.
4. Neutralize the trypsin using 0.1 ml Preadipocyte Medium (cat# PM-1) per cm² cultureware surface area (7.5 ml for T-75 flask). Check under a microscope to ensure all cells are liberated.
5. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and suspend cells in a volume of PM-1 appropriate for counting the cells. Count using a hemacytometer.
6. Centrifuge at 280 x g, 20°C, 5 minutes. Suspend in cold cryopreservation medium at a concentration of 1X10⁶ cells/ml. Do not exceed a 6:1 ratio of cells (per million): volume cryopreservation medium (per ml). Remember to account for the volume of the cell pellet before adding the volume of cryopreservation medium necessary for cell suspension.
7. If using a controlled-rate freezer: Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C. If using a cell cryopreservation container, prepare according to the manufacturer's instructions.
8. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

THAWING PROCEDURE

1. Remove cells from liquid nitrogen and place immediately into a 37°C water bath with agitation. Be careful not to submerge the cap of the vial into water. Do not leave the vials in water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
2. Upon the thawing, add the cells to a sterile conical bottom centrifuge tube, containing 10 ml of Preadipocyte Medium (PM-1).
3. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and suspend cells in a volume of PM-1 appropriate for counting the cells. Count using a hemacytometer.
4. Centrifuge again at 280 x g, 20°C, 5 minutes. Suspend in appropriate volume of plating medium for your cell type.

Figure 1. Comparison of percentage viability and post-thaw recovery using Zen-Bio's FM-1 versus other standard freezing medium in human primary preadipocytes.



PRECAUTIONS

Cryopreservation medium is for "in vitro research use only". Not for household, clinical or diagnostic use. Normal precautions used in handling laboratory reagents should be followed.

ORDERING INFORMATION

Description

Cryopreservation Medium, 100 ml

Catalog

FM-1-100

Optional Products

Preadipocyte Medium, 500 ml

PM-1

PBS, 1 Liter

DPBS-1000

Trypsin-EDTA Solution, 100 ml

TRP-100