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Cryopreservation of Mammalian Cells (Suspension)

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ABSTRACT

A protocol for the preservation of suspension-type cells by freezing.

The protocol has been sucessfully used on:

- THP-1 monocytes

MATERIALS

NAME	CATALOG #	VENDOR
RPMI 1640 with L-glutamine	10040CV	Corning
MEM Nonessential Amino Acids	25025CI	Corning
Sodium Pyruvate	25000CI	Corning
Fetal Bovine Serum	S1620-50	BioWest

BEFORE STARTING

Formulate and warm cryopreservation media consisting of:

- 50% fetal bovine serum
- 40% culture medium (may contribute additional FBS)
- 10% DMSO

1 Measure the density and viability of the cells

TC20 Automated Cell Counter
automated cell counter
BioRad 1450102

2 Gently pellet the cells via centrifugation

250 x g 00:07:00

3 While centrifuging, use the **live** cell counts from **step 1** to calculate the total volume required to resuspend the cells @

[M]2500000 cells/mL

4 Gently aspirate the culture media from the pellet

5 Gently reuspend the cell pellet in cryopreservation media:

- [M]50 Volume Percent fetal bovine serum
- [M]40 Volume Percent cell culture media
- [M]10 Volume Percent DMSO

Use the media volume calculated in **step 3**

6 Aliquot cells into labeled cryo vials

1 ml

7 Place cryo vials into a **room temp** Mr. Frosty freezing container

Mr. Frosty Freezing Container
freezing container
ThermoFisher Scientific 5100-0001

8 Freeze the container overnight

-80 °C 16:00:00 **(ie. overnight)**

9 Transfer the vials to liquid nitrogen for long term storage

10 Suggested cell culture media for **step 5**

step case

THP-1s

RPMI-1640, supplemented with:
- sodium pyruvate (1 mM)
- non-essential amino acids (1x)
- fetal bovine serum (10%)

step case

THP-1s

RPMI-1640, supplemented with:

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