



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

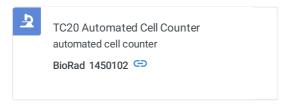
MATERIALS

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
- Measure the density and viability of the cells



Gently pellet the cells via centrifugation

3250 x g (300:07:00)

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 DMS0
	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
	8 -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**

THP-1s

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

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