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Protocol status: Working
 We use this protocol and it's working

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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 successfully used on:

- THP-1 monocytes

MATERIALS

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RPMI 1640 with L-glutamine **Corning Catalog #10040CV**

MEM Nonessential Amino Acids **Corning Catalog #25025CI**

Sodium Pyruvate **Corning Catalog #25000CI**

Fetal Bovine Serum **BioWest Catalog #S1620-50**

BEFORE START INSTRUCTIONS

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

1

Equipment	
TC20 Automated Cell Counter	NAME
automated cell counter	TYPE
BioRad	BRAND
1450102	SKU
http://www.bio-rad.com/en-us/product/tc20-automated-cell-counter?ID=M7FBG34VY&WT.mc_id=170125000484&WT.srch=1&WT.knsh_id=a8d381b8-4072-42a1-9b46-e12b739c51d1&gclid=CjwKCAjw6vvoBRBtEiwAZq-T1bzXM1YIstQFme2X7mMBPmH32OVGbPi9d7Gf5ZDnbv3PEjrzFqcwbxoCqM8QAvD_BwE	LINK

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 resuspend the cell pellet in cryopreservation media:

- **[M] 50 % volume** fetal bovine serum
- **[M] 40 % volume** cell culture media
- **[M] 10 % volume** 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

Equipment	
Mr. Frosty Freezing Container	NAME
freezing container	TYPE
ThermoFisher Scientific	BRAND
5100-0001	SKU
https://www.thermofisher.com/order/catalog/product/5100-0001	LINK

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 10 includes a Step case.

THP-1s