Jul 28, 2021

CELL STORAGE-02-Freezing and Thawing Protocol for Suspension Cell Lines

Marco Cosentino[1], Alessandra Luini[1], Massimiliano Legnaro[1], Emanuela Rasini[1], Mariagiulia Albizzati[1], Marco Ferrari[1], Franca Marino[1], Alessandra Luini[1], Alessandra Luini[1]

[1]Center for Research in Medical Pharmacology, University of Insubria (Varese, Italy)

dx.doi.org/10.17504/protocols.io.bvwxn7fn

Farmacologia Medica

Citation: Marco Cosentino, Alessandra Luini, Massimiliano Legnaro, Emanuela Rasini, Mariagiulia Albizzati, Marco Ferrari, Franca Marino, Alessandra Luini, Alessandra Luini (07/28/2021). CELL STORAGE-02-Freezing and Thawing Protocol for Suspension Cell Lines. https://dx.doi.org/10.17504/protocols.io.bvwxn7fn

ABSTRACT

In our institute, cell lines are stored after freezing procedure that provides for a constant temperature lowering by 1 degree/minute. This procedure is useful to minimizes ice crystals formation during freezing process, this allow to reduce the cells damage and, in turn increasing cells viability after thawing. After storage at -80°C or in liquid nitrogen (in relation to subsequent applications or the expected freezing time) cells are thawed rapidly in a water bath at 37°C and the cryopreserving-medium is immediately remove by gentle centrifugation in order to reduce as soon as possible, the DMSO toxic effect.

Currently, in our institute, the freezing/thawing procedure is validated for the following cell lines:

- **PC-12**, Rat adrenal phaeochromocytoma, small irregularly shaped cells morphology, suspension growth mode.

After freezing/thawing procedure, cells quality must be assessed by cell morphology evaluation (using Optical Microscopy technique) and time required to reach confluence in the T25 cm2 culture flask, which must not exceed the times indicated in the **Table 1**.
After quality test cells must be resuspended according to specific protocols (for details see Table 2).

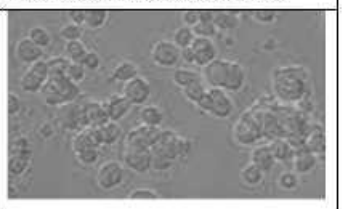
Cell lines	Time to Reach Confluence	Reference for morphology evaluation
PC12	72 hours	

Table 1

Cell·line¤	Culture·medium¤	Applications·post·thawing¤	Our·publication·using·this·applications¤
PC12¤	RPMI·1640+5%FBS+10%·FHS+100·U/ml·penicillin/streptomycin¤	Cell·cultures¤	Cosentino·et·al.,·2019¤

¶

Table 2

List of published work by our institute that using this procedure

- Cosentino M, Marino F, Rasini E, Legnaro M, Bombelli R, Luini A, Pacchetti B. **Improved solubility and increased biological activity of NeoSol™RCL40, a novel Red Clover Isoflavone Aglycones extract preparation.** Biomed Pharmacother. 2019 Mar;111:91-98. doi: 10.1016/j.biopha.2018.12.065. Epub 2018 Dec 19. PMID: 30579257.

DOI

dx.doi.org/10.17504/protocols.io.bvwxn7fn

Citation: Marco Cosentino, Alessandra Luini, Massimiliano Legnaro, Emanuela Rasini, Mariagiulia Albizzati, Marco Ferrari, Franca Marino, Alessandra Luini, Alessandra Luini (07/28/2021). CELL STORAGE-02-Freezing and Thawing Protocol for Suspension Cell Lines. https://dx.doi.org/10.17504/protocols.io.bvwxn7fn

PROTOCOL CITATION

Marco Cosentino, Alessandra Luini, Massimiliano Legnaro, Emanuela Rasini, Mariagiulia Albizzati, Marco Ferrari, Franca Marino, Alessandra Luini, Alessandra Luini 2021. CELL STORAGE-02-Freezing and Thawing Protocol for Suspension Cell Lines. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvwxn7fn

CREATED

Jun 17, 2021

LAST MODIFIED

Jul 28, 2021

PROTOCOL INTEGER ID

50871

MATERIALS TEXT

Reagents

- **FBS** catalog number: ECS0180L-500 ml, Euroclone
- **RPMI 1640** catalog number: ECM0495L-500 ml, Euroclone
- **FHS** catalog number: ECS0090L-500 ml, Euroclone
- **Penicillin/streptomycin** catalog number: ECB3001D-500 ml, Euroclone
- **DMSO** catalog number 276855, Sigma-Aldrich

Sterile plastic disposable:

- **Cryogenic vials** catalog number: CC430659, Corning
- **T25 cm^2 culture flasks for suspension cells** catalog number: CC431463, Corning

Instrumentation required:

- Laminar flow hood
- Centrifuge
- -80°C freezer

CELL FREEZING PROCEDURE FOR SUSPENSION CELLS (PC-12)

1 At confluence remove cell suspension from the T25 cm^2 culture flask and recover in 15 mL conical tube.

2 Centrifuge for **00:05:00** at **200 x g** **Room temperature** .

3 Remove supernatants and resuspend the cell pellet in **1 mL** of cryopreserving medium (90% FBS + 10% DMSO).

4 Immediately aliquot **1 mL** of cell suspension into a cryogenic vial.

5 Place the cryogenic vial(s) in cotton wool, and then place it in a polystyrene box.

6 Put the polystyrene box in -80°C freezer.

-80°C Freezer

Eppendorf **B U9230-0001**

7 After a minimum of **24:00:00** transfer the cryogenic vials into a box for storage in -80°C freezer. 1d

CELL THAWING PROCEDURE 5m

8 Remove cryogenic vial from -80°C and keep it at **37 °C** in water bath, this procedure should take approximately 1-2 minutes or until ampoule is slightly thawed.

9 Clean the cryogenic vial outside with 70% EtOH, open the cryogenic vial and add **1 mL** of prewarmed complete medium for specific cells lines (**Table 2**).

10 Transfer the cell suspension into a 15 mL conical tube containing **10 mL** of prewarmed complete medium for specific cells lines.

11 Centrifuge for **00:05:00** at **200 x g** **Room temperature** . 5m

12 Resuspend the cells into appropriate cell culture medium (**Table 2**) and start quality control procedure (**Table 1**).

Citation: Marco Cosentino, Alessandra Luini, Massimiliano Legnaro, Emanuela Rasini, Mariagiulia Albizzati, Marco Ferrari, Franca Marino, Alessandra Luini, Alessandra Luini (07/28/2021). CELL STORAGE-02-Freezing and Thawing Protocol for Suspension Cell Lines. https://dx.doi.org/10.17504/protocols.io.bvwxn7fn