Video Article

Freezing Human ES Cells

Erin Trish[1], John Dimos[1], Kevin Eggan[1]

[1]Department of Molecular and Cell Biology, Harvard

Correspondence to: Erin Trish at etrish@mcb.harvard.edu, John Dimos at jdimos@fas.harvard.edu, Kevin Eggan at eggan@mcb.harvard.edu

URL: https://www.jove.com/video/50
DOI: doi:10.3791/50

Keywords: Cellular Biology, Issue 1, Embryonic Stem Cells, ES, Tissue Culture, Freezing

Date Published: 10/12/2006

Citation: Trish, E., Dimos, J., Eggan, K. Freezing Human ES Cells. *J. Vis. Exp.* (1), e50, doi:10.3791/50 (2006).

Abstract

Here we demonstrate how our lab freezes HuES human embryonic stem cell lines. A healthy, exponentially expanding culture is washed with PBS to remove residual media that could otherwise quench the Trypsin reaction. Warmed 0.05% Trypsin-EDTA is then added to cover the cells, and the plate allowed to incubate for up to 5 mins at room temperature. During this time cells can be observed rounding, and colonies lifting off the plate surface. Gentle repeated pipetting will remove cells and colonies from the plate surface. Trypsinized cells are placed in a standard conical tube containing pre-warmed hES cell media to quench remaining trypsin, and then spun. Cells are resuspended growth media at a concentration of approximately one million cells in one mL of media, a concentration such that one frozen aliquot is sufficient to resurrect a culture on a 10cm plate. After cells are adequately resuspended, ice cold freezing media is added at equal volume. Cell suspensions are mixed thoroughly, aliquoted into freezing vials, and allowed to slowly freeze to -80C over 24 hours. Frozen cells can then moved to the vapor phase of liquid nitrogen for long term storage, or remain at -80 for approximately six months.

Video Link

The video component of this article can be found at https://www.jove.com/video/50/

Protocol

1. A healthy, exponentially expanding culture is washed with PBS to remove residual media that could otherwise quench the Trypsin reaction.
2. Warmed 0.05% Trypsin-EDTA is then added to cover the cells, and the plate allowed to incubate for up to 5 mins at room temperature. During this time cells can be observed rounding, and colonies lifting off the plate surface.
3. Gentle repeated pipetting will remove cells and colonies from the plate surface.
4. Trypsinized cells are placed in a standard conical tube containing pre-warmed hES cell media to quench remaining trypsin, and then spun.
5. Cells are resuspended growth media at a concentration of approximately one million cells in one mL of media.
6. After cells are adequately resuspended, ice cold Freezing Media is added at equal volume.
7. Cell suspensions are mixed thoroughly, aliquoted into freezing vials, and allowed to slowly freeze to -80C over 24 hours.
8. Frozen cells can then moved to the vapor phase of liquid nitrogen for long term storage, or remain at -80 for approximately six months.

Note: such a frozen aliquot at a concentration of 106 cells/ml is sufficient to resurrect a culture on a 10cm plate.

Discussion

The technique we describe for freezing HuES cell lines works well for efficiently storing and resurrecting HuES cell lines and also any mammalian cell of interest. Freezing a small proportion of cells at each passage is an important consideration when working with hES cell lines, as karyotype abnormalities can arise and abnormal cells can quickly take over the line. Thus, having frozen splits at every passage in addition to large banks at certain passages is highly desirable.

References

1. Cowan, C.A. et. al, Derivation of Embryonic Stem Cells from Human Blastocysts (2004), NEJM 1997; 336(23):1650-1656.

