Video Article

Human ES cells: Starting Culture from Frozen Cells

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Abstract

Here we demonstrate how our lab begins a HuES human embryonic stem cell line culture from a frozen stock. First, a one to two day old ten cm plate of approximately one (to two) million irradiated mouse embryonic fibroblast feeder cells is rinsed with HuES media to remove residual serum and cell debris, and then HuES media added and left to equilibrate in the cell culture incubator. A frozen vial of cells from long term liquid nitrogen storage or a -80C freezer is sourced and quickly submerged in a 37C water bath for quick thawing. Cells in freezing media are then removed from the vial and placed in a large volume of HuES media. The large volume of HuES media facilitates removal of excess serum and DMSO, which can cause HuES human embryonic stem cells to differentiate. Cells are gently spun out of suspension, and then re-suspended in a small volume of fresh HuES media that is then used to seed the MEF plate. It is considered important to seed the MEF plate by gently adding the HuES cells in a drop wise fashion to evenly disperse them throughout the plate. The newly established HuES culture plate is returned to the incubator for 48 hrs before media is replaced, then is fed every 24 hours thereafter.

Video Link

The video component of this article can be found at https://www.jove.com/video/86/

Protocol

Thaw from a frozen stock:

1. Before thawing HuES cells, ensure that the MEF plate you have already prepared is properly plated and in good condition. DO NOT try to use a less than desirable plate, or one that is older than three days. It is recommended to pre-label all conical tubes and wells being used.
2. Pre-warm HuES media to 37°C. Aliquot HuES media into a sterile conical tube for each line. Remove HuES vial/s from -80 and immediately submerge the bottom half of the tube in a 37°C water bath. It should take about 45-60 seconds before the cells are 80% thawed (small frozen portion left).
3. Quickly bring the tube to the laminar flow hood, spray down with 70% alcohol, and gently transfer cells to the of pre-warmed media. Spin the tube, remove media, and resuspend in an appropriate volume of fresh, pre-warmed HuES media.
4. Aspirate off the MEF media from as many wells as you will be thawing into. Quickly, aliquot pre-warmed HuES media back into each well of the plate, being careful not to disturb the attached MEFs. Set the plate aside in the hood. As with MEFs, best results are obtained if the drops are evenly distributed about the plate. Carefully return the plate to a 37°C incubator overnight to allow the HuES cells to seed the MEFs. Change media ~48 hrs after thaw, replacing with fresh HuES media.

Discussion

In this video, we show how our laboratory routinely establishes a HuES human embryonic stem cell culture from a frozen stock. This process is amenable for general use with any mammalian cell if frozen in a DMSO solution. Efficient thawing is essential for establishing a new culture of HuES cells from a frozen stock, and is important for experimental consistency.

Disclosures

The authors have nothing to disclose.

References

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

