invitrogen™

# Points to consider

## when culturing human Embryonic Stem Cells with STEMPRO® hESC SFM

- Starter culture
    - This must be a high quality culture. There must be a high density of cells, and they must be primarily undifferentiated. The starter culture should be cells maintained on Geltrex™ in MEF-CM, i.e., not hESCs on MEFs.

- Passaging
    - Passaging the cells is the most likely point of difficulty. It is absolutely critical to achieve high plating/survival of colony pieces. The colony pieces must be a bit smaller than typical collagenase passaging of cells on Geltrex™/MEF-CM .
    - Some cell death at passaging is normal, but wide-scale cell death is not (ie <20% survival), and typically indicates a poor split.

- Timing of passaging
    - This is critical. Do not passage the cells too early, they will plate poorly and differentiate. The cultures need to grow to near-confluence, i.e., a day or two longer than when the colonies are just touching. We routinely harvest 5-8 million cells per 60 mm dish.

- hESCs in STEMPRO® hESC SFM are very sensitive to over exposure to collagenase. This will cause poor plating and induce differentiation. Do not expose longer than 3 minutes. Do not use lower concentrations of collagenase and treat for longer periods.

- Density
    - The cultures must be maintained at a high density. This means 200+ colonies in a 60 mm dish. If the density of colonies drops, the culture will tend to deteriorate: cells will differentiate, and the culture will take longer between splits. If this happens, leave the culture longer to proliferate to near-confluence before splitting.

- The "knife edge"
    - Just as with hESCs grown in other conditions, cells in STEMPRO® hESC SFM sit on a knife edge of proliferation vs. differentiation. The cultures should be fed every day. Do not exhaust the medium by not feeding. Badly differentiated areas should be scraped out with a pipette tip. Mistreated cultures will differentiate.

    - Passage the cells 2-3 times in the previous ratio of MEF conditioned media to defined media.

- We strongly recommend that you always take these precautions
    - Make a frozen stock of the cells in MEF conditioned media prior to adaptation
    - Keep a culture going of the cells in each prior condition when starting the next level of adaptation as a fall-back if the cells do not survive in the next passage.

- Technical support
    - Please contact Tech Services at Invitrogen
      United States TECH-LINE℠: 1 800 955 6288
      Canada TECH-LINE℠: 1 800 757 8257

      Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

- You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

# References

In this section we list references to articles that may further help you culture and understand human Embryonic Stem Cells.

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