Two independent studies demonstrate improved growth characteristics for hESCs in DMEM/F-12 Low Osmolality medium

The two studies, one at the University of California, Irvine, and the other at the Buck Institute for Age Research, revealed that human embryonic stem cells (hESCs) grown in Invitrogen's DMEM/F-12 Low Osmolality exhibit:

- → Accelerated growth
- → Normal morphology and karyotype
- → Pluripotency
- → Little to no spontaneous differentiation
- → Equivalent morphology to manually passaged hESCs, even after enzymatic passaging

Study 1: University of California, Irvine

DMEM/F-12 Low Osmolality enables faster growth of hESCs with normal morphology and little or no spontaneous differentiation.

This study investigated the growth characteristics of hESC cultures in the following 3 media formulations* (Figure 1):

- → Conditioned medium control
- → DMEM/F-12
- → DMEM/F-12 Low Osmolality

The UC Irvine study also examined changes in morphology and differentiation of stem cells grown in three types of media. hESCs grown in DMEM/F-12 Low Osmolality exhibited normal morphology and little spontaneous differentiation, compared to colonies grown in other media (Figure 2).

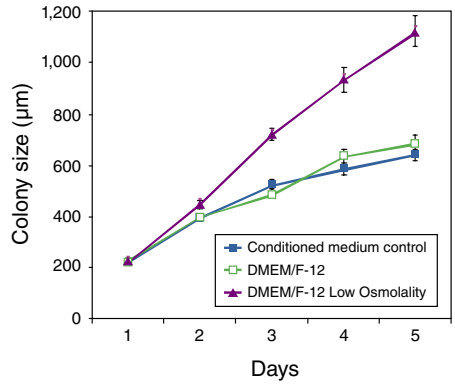

Figure 1—Growth rate of hESC colonies is enhanced using DMEM/F-12 Low Osmolality. The stem cell colony diameters were measured over a passage interval of 5 days.

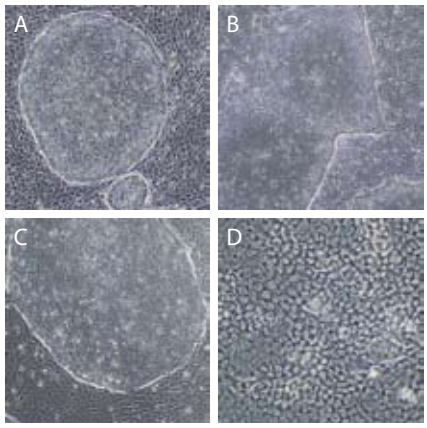

Figure 2—Normal morphology, little differentiation of hESCs in DMEM/F-12 Low Osmolality. **A.** hESC colonies grown in Knockout™ DMEM and Knockout™ Serum Replacement, incubated for 24 hr on mouse embryonic feeders. **B.** hESC colonies grown in DMEM/F-12. Marginal tendency for differentiation is observed. **C and D.** hESC colonies grown in DMEM/F-12 Low Osmolality. Colonies displayed normal morphology (smooth, homogeneous surface, well delimited, single-layered) with little or no tendency to spontaneously differentiate.

* Media formulations contain supplements and growth factors, details of which are not disclosed in this report.

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Study 2: Buck Institute of Age Research

Human ESCs grown in DMEM/F-12 Low Osmolality retain pluripotency and normal morphology the most, even with enzymatic passaging

This study investigated the growth characteristics of hESCs on two media formulations:

→ Mouse embryonic fibroblast (mEF) feeder layer in DMEM/F-12 Low Osmolality (Figure 3)

→ DMEM/F-12 Low Osmolality media conditioned with mEF (Figure 4)

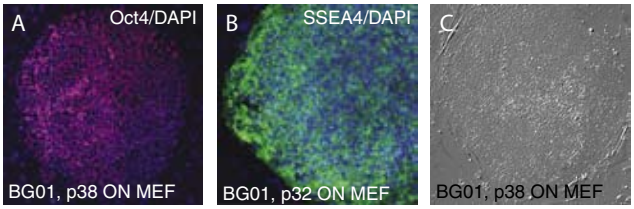

Figure 3—hESCs grown on a layer of mEFs in DMEM/F-12 Low Osmolality with enzymatic passaging exhibit normal morphology and 46, XY karyotype. hESCs were continuously passaged by collagenase in reduced osmolality medium for 7 passages and expressed pluripotency markers Oct4 and SSEA4. The morphology of these cells is comparable to manually passaged hESCs. **A.** 10× magnification for anti-Oct4 staining. **B.** 20× magnification for anti-SSEA4 staining **C.** Phase-contrast image of a representative hESC colony. .

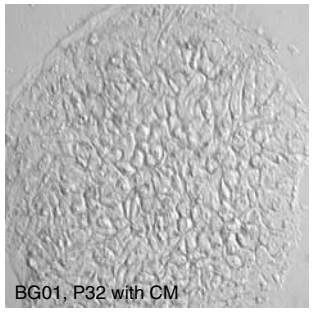

Figure 4—hESCs continuously passaged by collagenase in conditioned medium for 4 passages. Cells exhibited excellent morphology and very clear boundaries. A few differentiating cells were observed between the colonies (20× magnification).

Ordering information

Product	Quantity	Cat. no.
DMEM/F-12 Low Osmolality (1X), liquid, 1:1	500 ml	12660-012

For a complete review of Invitrogen stem cell technologies and services, please visit www.invitrogen.com/stemcell.