Serum- and feeder-free media

StemPro® hESC SFM

Human Embryonic Stem Cell Culture Medium

invitrogen™

StemPro® hESC SFM—a fully defined, serum- and feeder-free medium (SFM) specially formulated for the growth and expansion of human embryonic stem cells (hESCs)

- → Enables superior expansion of hESCs
- → Maintains pluripotency, normal morphology/karyotype, and differentiation capability of hESCs
- → Provides gene expression profiles comparable to classical media
- → Each lot is performance qualified using PCR
- → No or little adaptation required from feeder cultures

Serum- and feeder-free hESC culture

Advances in hESC research are reported daily, shedding more light on how hESCs, which are the building blocks for all cell types in the human body, may be harnessed for potential applications in cell therapy and regenerative medicine. Currently, hESC culture and expansion methods require the use of serum, mouse or human fibroblast feeder layers, or feeder-conditioned medium.[1] These culture methods are labor intensive and hard to scale, and it is difficult to maintain hESCs in an undifferentiated state because of significant sources of variability such as growth factor fluctuations during culture.

Invitrogen has developed StemPro® hESC SFM,[2] which provides a breakthrough solution to these problems by enabling serum- and feeder-free culture of hESCs. StemPro® hESC SFM is prequalified for hESCs and has cGMP-manufactured components that give you the quality and consistency needed to optimize your hESC culture.

StemPro® hESC SFM has been extensively tested and proven to maintain pluripotency in a growing list of hESC lines including: BG01, BG02, BG03, H1, H9, HUES9, and BG01V. For an up-to-date list of hESC lines that StemPro® hESC SFM has been tested on, please visit www.invitrogen.com/StemPro/hESC. StemPro® hESC SFM is the best pick when selecting a defined media for hESC culture as other defined media either give rise to cells with an abnormal karyotype[3] or still require the use of feeder layers.

StemPro® hESC SFM enables superior hESC expansion

hESC expansion is a critical step in the hESC workflow in order to generate enough cells for subsequent steps such as engineering, differentiation, and transplantation. Superior BG03 cell expansion is achieved using StemPro® hESC SFM compared to expansion using MEF-Conditioned Media (MEF-CM; control) (Figure 1). Furthermore, BG01 and BG01V cells grown in StemPro® hESC SFM exhibit higher yields compared to MEF-CM, i.e., allow higher split ratios with similar passaging frequencies versus controls (Table 1).

The ability to scale up production of hESCs will be important for any cell therapy to be viable. StemPro® hESC SFM was used to scale up BG02 cells to 1.2×10^{10} cells starting from 1×10^{6} cells over a 4-week period (Table 2). Furthermore, BG02 cells grown in StemPro® hESC SFM can be expanded for >50 passages.

StemPro® hESC SFM maintains pluripotency and normal morphology/karyotype

BG02 cells grown in StemPro® hESC SFM for >50 passages exhibit normal morphology where the multicellular hESC colonies exhibit a high nucleus-to-cytoplasm ratio and prominent nucleoli. (Figure 2).

hESCs are defined by their ability to proliferate indefinitely while retaining an undifferentiated state. In order to assess whether hESCs are still in an undifferentiated state, characterization[4] is performed to determine the presence of specific cell surface markers such as stage specific embryonic antigens (SSEA-3 and SSEA-4), tumor rejection antigen 1 (TRA-1-60 and TRA-1-81), and expression of certain genes such as the transcription factor Oct4. Characterization by flow cytometry, RT-PCR, and immunofluorescence of BG02 cells grown in StemPro® hESC SFM showed maintenance of pluripotency (Figure 3).

Cytogenetic analysis of BG01 and BG02 cells grown in StemPro® hESC SFM at low and high passage numbers shows that a normal karyotype is retained (Figure 4). This is contrary to other defined media that give rise to hESCs with an abnormal karyotype[3] and hence demonstrates why StemPro® hESC SFM is the best choice when selecting a defined media for hESC culture.

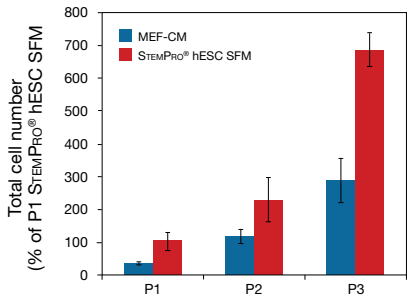


Figure 1—StemPro® hESC SFM provides a 140% improvement in expansion over 3 passages compared to MEF-CM. BG03 cells growing on MEFs were passaged directly into either StemPro® hESC SFM or MEF-CM. The cultures were split in parallel every 4 days. Triplicate wells were counted and cumulative total cell number plotted as a percentage of the total cell number after 1 passage (P1) in StemPro® hESC SFM.

Table 1—StemPro® hESC SFM allows higher split ratios with similar passaging frequencies for BG01 and BG01V cells versus control (MEF-CM).

Medium	Passaging frequency (days)	Split ratio during passaging
MEF-CM	3–4	1:3
StemPro® hESC SFM	3–4	1:4–5

Table 2—Scale-up of BG02 cells using StemPro® hESC SFM over a 4-week period.

Time	Container	Media	Yield
Day 0	1 × 60	20 ml total	~8×10^{6}
Day 6	4 × 60	80 ml total	~6×10^{7}
Day 12	3 × T175	525 ml total	~3×10^{8}
Day 18	8× triple flask	4 L	~2×10^{9}
Day 24	50× triple flask	25 L	~1×10^{10}

Total media requirements = 30 L (includes daily media changes)

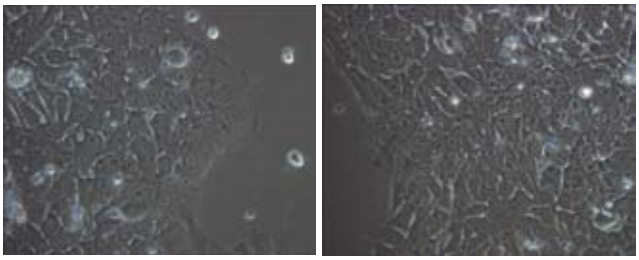


Figure 2—hESCs grown in StemPro® hESC SFM for >50 passages retain normal morphology. Phase contrast images (40×) of BG02 cells grown in StemPro® hESC SFM (passage 55).

A

hESC line	Total number of passages	Number of passages of StemPro® hESC SFM	Karyotype
BG01	32	6	46, XY
	41	18	46, XY
BG02	47	5	46, XY
	61	19	46, XY
	71	26	46, XY
	89	45	46, XY

B

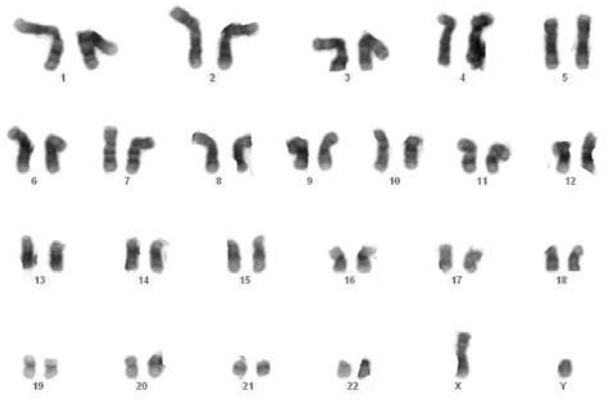
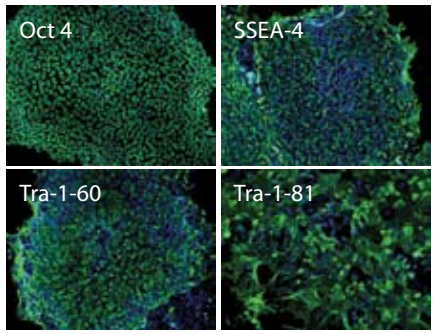


Figure 4—hESCs grown in StemPro® hESC SFM retain normal karyotype. **A.** Total passage number, passages in StemPro® hESC SFM, and karyotype. **B.** Example of G-banding of BG02 cells grown in StemPro® hESC SFM p26 cells.

A



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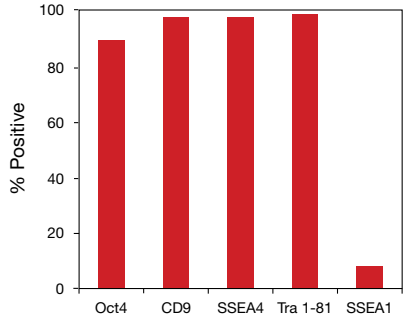

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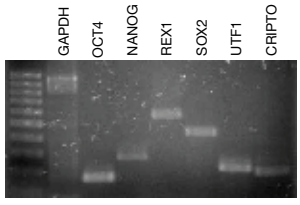

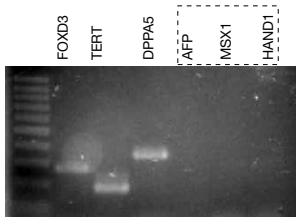

Figure 3—hESCs grown in StemPro® hESC SFM express pluripotency markers. **A.** Immunofluorescence analysis of BG02 cells (passage 8) shows expression of Oct4, SSEA-4, Tra-1-60, and Tra-1-81. The green fluorescence is specific for the respective antibody and the blue is a counter stain by DAPI. **B.** Flow cytometry analysis of BG02 cells scaled up in StemPro® hESC SFM shows expression of pluripotency markers. **C.** BG02 cells scaled up in StemPro® hESC SFM express pluripotency markers but do not express differentiated cell markers as shown by RT-PCR.

StemPro® hESC SFM maintains pluripotent differentiation capability of hESCs

hESCs are also defined by their ability to differentiate into cells of the three embryonic germ layers.[5] Differentiation of hESCs can be studied in vivo through the induction of teratomas in immune-deficient mice. Cells within the teratomas differentiate into all three embryonic germ layers: ectoderm, mesoderm, and endoderm. Teratoma analysis of BG02 cells grown in StemPro® hESC SFM demonstrates the maintenance of differentiation capabilities into all three germ lineages (Figure 5).

StemPro® hESC SFM maintains the gene expression profile of hESCs

It is important that a new culture medium does not significantly alter the genetic profile of cells as compared to traditional media. To confirm that StemPro® hESC SFM maintains the standard genetic profiles, Illumina BeadArray™ technology was used to compare the gene expression profiles of cells grown in StemPro® hESC SFM with those grown in MEF-CM. Similar gene expression is obtained when hESCs are grown in either medium (Figure 6).

Each StemPro® hESC SFM Kit includes StemPro® hESC Supplement, Bovine Serum Albumin 25% (BSA), and DMEM/F-12 with GlutaMAX™ (Figure 7).

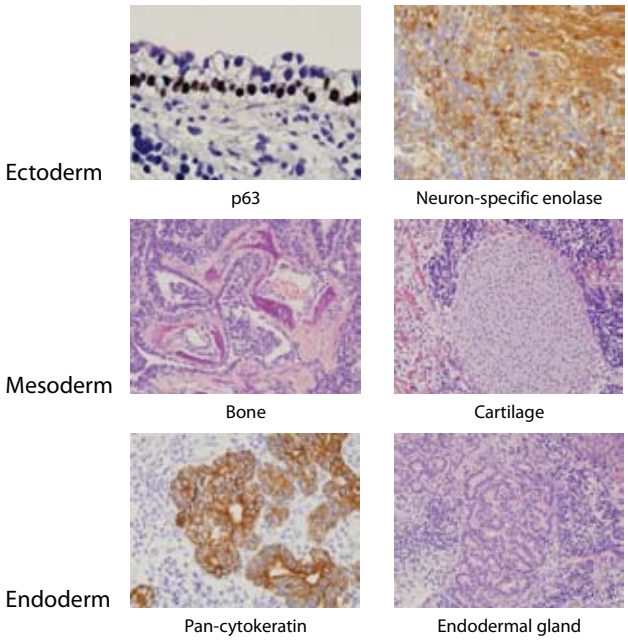

Figure 5—hESCs grown in StemPro® hESC SFM differentiate into all 3 germ line lineages. Analysis of teratomas from BG02 cells grown in StemPro® hESC SFM at p25 demonstrated pluripotent differentiation potential to ectoderm, mesoderm, and endoderm.

References

1. Thomson, J.A. et al. (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145.
2. Wang, L.. et al. (2007) Self-renewal of Human Embryonic Stem Cells Requires Insulin-Like Growth Factor-1 Receptor and ERBB2 Receptor Signaling. *Blood* (In Press).
3. Ludwig, T.E. et al. (2006) Derivation of human embryonic stem cells in defined conditions. *Nat Biotechnol* 24: 185–7.
4. Hoffman, L.M. and Carpenter, M.K. (2005) Characterization and culture of human embryonic stem cells. *Nat Biotechnol* 23: 699–708.
5. Pal, R. et al. (2007) A panel of tests to standardize the characterization of human embryonic stem cells. *Regen Med* Mar 2(2): 179–92.

To learn more, ask your local Invitrogen Account Manager or visit www.invitrogen.com/StemPro/hESC for product information, protocols, and an up-to-date list of hESC lines that StemPro® hESC SFM has been tested on.

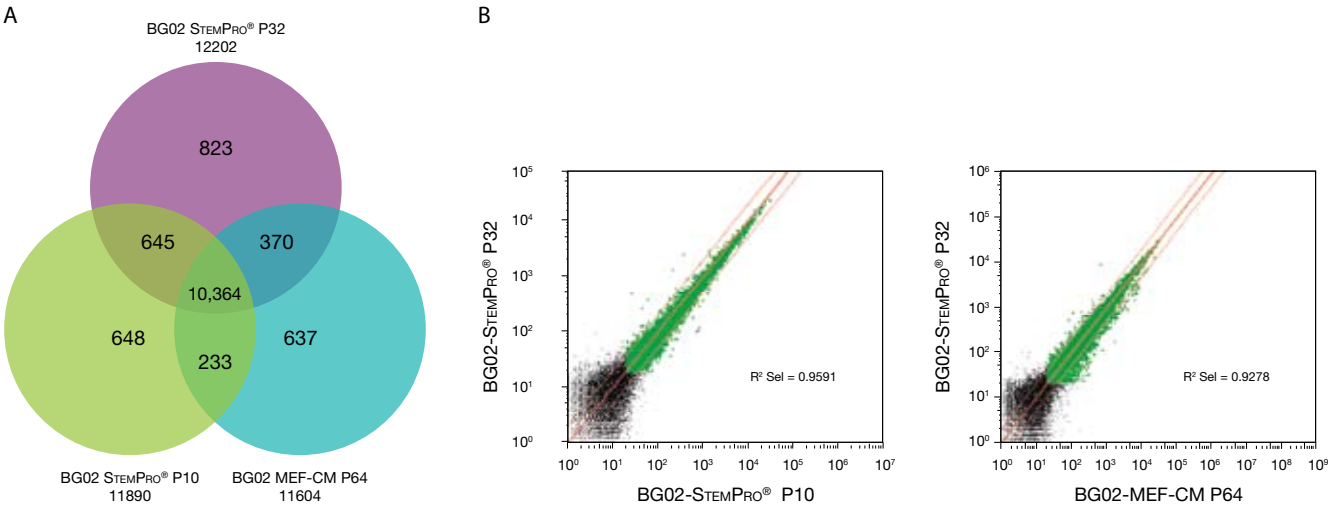

Figure 6—StemPro® hESC SFM maintains global mRNA expression of hESCs. **A.** Venn diagram of the distribution of transcripts detected using high-density Illumina Sentrix® Human-6 Expression BeadChip containing 47,296 transcript probes in BG02 cells maintained in MEF-CM (control; 64 passages) or StemPro® hESC SFM (10 or 32 passages in StemPro®). A large proportion of the expressed transcripts were detected in all samples. **B.** Scatterplot analysis demonstrated that the transcriptional profile of BG02 cells maintained in StemPro® hESC SFM was highly similar to that of BG02 cells maintained in MEF CM (control; right), and was not substantially altered in early and late passage cultures in StemPro® hESC SFM (left). Correlation coefficient (R^2 select) was generated using transcripts exhibiting a detection confidence level of >0.99 (green dots). Red lines delineate the mean and limits of a 2-fold difference.

Figure 7—StemPro® hESC SFM Kit.

Advance your hESC research with fully defined, serum- and feeder-free media. Use StemPro® hESC SFM.

Ordering information

Product	Quantity	Cat. no.
StemPro® hESC SFM	1 kit	A10007-01

StemPro® hESC SFM is shipped in two parts with separate storage requirements and catalog numbers:
1. StemPro® hESC Supplement (10 ml, Cat. no. A10006-01)
2. Bovine serum albumin 25% (BSA) (40 ml, Cat. no. A10008-01) and DMEM/F-12 with GlutaMAX™ (500 ml, Cat. no. 10565-018).

These components, with the exception of DMEM/F-12 with GlutaMAX™, are not sold individually. For additional information and to place your order, please visit www.invitrogen.com/StemPro/hESC and www.invitrogen.com.

Note: Additional componets required for complete medium: bFGF (full length) REC HU (Cat. no. PHG0026) and 2-mercaptoethanol (Cat. no. 21985-023) need to be purchased separately.