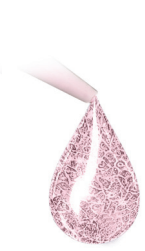




First xeno-free serum replacement for pluripotent stem cells

KnockOut™ SR XenoFree





New, xeno-free growth and expansion of hESCs and human iPSCs

KnockOut™ SR XenoFree

- Equivalent to traditional KnockOut™ SR (serum replacement) with little or no adaptation required
- Supports derivation and routine maintenance of hESCs and human iPSCs
- Maintains pluripotency, normal morphology, and karyotype of hESC
- Retains pluripotent gene expression and differentiation capability
- Use with feeder cells or in a feeder-free environment with CELLstart™ and KnockOut™ SR GF Cocktail
- Formulated with human-derived or human recombinant proteins under cGMP conditions

KnockOut™ SR XenoFree

Advances in human embryonic stem cell (hESC) and human induced pluripotent stem cell (iPSC) research are shedding light daily on how these cells can build the body's 200 cell types. Human iPSCs are a potential source of patient-specific pluripotent stem cells that would not elicit an immune response. Human iPSCs can also be used to study diseases that have no adequate human *in vitro* or animal models. Harnessing the potential of these cells holds promise for future applications for cell therapy and regenerative medicine. Currently, hESC and human iPSC culture and expansion require serum, mouse or human fibroblast feeder layers, or feeder-conditioned medium.¹⁻³ These methods are labor intensive and hard to scale, and sources of variability like growth factor fluctuations during culture make it difficult to maintain hESCs and human iPSCs in an undifferentiated state.

Animal-derived components in culture media and animal-origin feeder cells can contaminate hESCs and human iPSCs with animal proteins during derivation, passaging, expansion, and cryopreservation. Patients are therefore at risk for animal pathogen cross-transfer. New animal origin-free products must be introduced to advance this important research into the clinic.

GIBCO® KnockOut™ SR XenoFree enables hESC and human iPSC growth and expansion in media containing only human-derived or human recombinant proteins, facilitating the transition of hESC research from the bench to the clinic. KnockOut™ SR XenoFree does not contain animal-derived components. Besides routine maintenance of hESCs and human iPSCs, KnockOut™ SR XenoFree can be used for hESC/human iPSC derivation, cryopreservation, embryoid body formation, and *in vitro* differentiation studies.

Performance equivalent to KnockOut™ SR

This new formulation is based on traditional KnockOut™ SR, used for more than 10 years in hESC labs worldwide. Equivalent hESC growth rates are observed when comparing KnockOut™ SR to KnockOut™ SR XenoFree (Figure 1).

For xeno-free culture using human foreskin fibroblast (HFF) feeder cells, tissue culture treated vessels can be coated with CELLstart™ humanized substrate prior to plating HFF in complete medium. Once HFF feeder cells have attached and spread, hESCs can be plated on HFF vessels. KnockOut™ SR XenoFree and

CELLstart™ substrate also support feeder-free hESC growth when supplemented with bFGF and KnockOut™ SR GF Cocktail.⁴

Supports hESC cryopreservation and maintains normal morphology/karyotype

hESCs grown in KnockOut™ SR XenoFree without feeder cells for 15 passages were cryopreserved and subsequently recovered in KnockOut™ SR XenoFree without feeder cells (Figure 2). Cytogenetic analysis of CyT49 cells grown in KnockOut™ SR XenoFree shows retention of a normal karyotype (Figure 3).

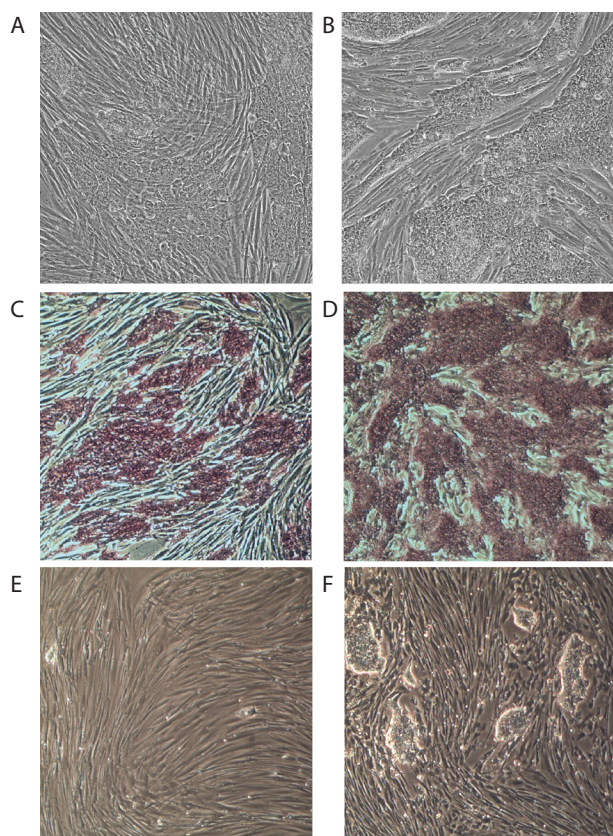


Figure 1. hESCs grown in KnockOut™ SR and KnockOut™ SR XenoFree on human foreskin fibroblasts (HFF) attached with CELLstart™ substrate exhibit similar growth characteristics. Phase contrast of BG01v cells cultured in either (A) KnockOut™ SR or (B) KnockOut™ SR XenoFree (passage 4). Alkaline phosphatase staining of BG01v cells cultured in (C) KnockOut™ SR or (D) KnockOut™ SR XenoFree (passage 5). Phase contrast of BG01v cells cryopreserved after 10 passages in (E) KnockOut™ SR or (F) KnockOut™ SR XenoFree and recovered in the same medium (day 3 post-thaw).

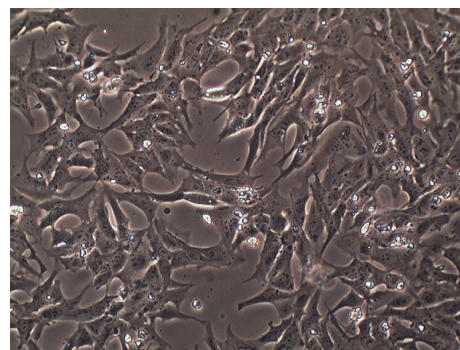


Figure 2. Morphology of feeder-free hESCs cultured in KnockOut™ SR XenoFree post-recovery from liquid nitrogen. BG01v cells were cultured in KnockOut™ SR XenoFree Medium containing KnockOut™ SR GF Cocktail on CELLstart™ substrate for 15 passages and then cryopreserved. Phase contrast image of BG01v cells on day 2 post-recovery.

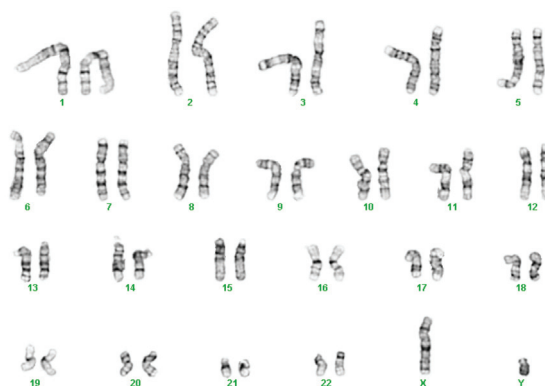


Figure 3. CyT49 cells grown in KnockOut™ SR XenoFree retain normal karyotype. G-banding of CyT49 cells grown in KnockOut™ SR XenoFree at passage 12. Data provided by Tom Schulz at Novocell, Inc.

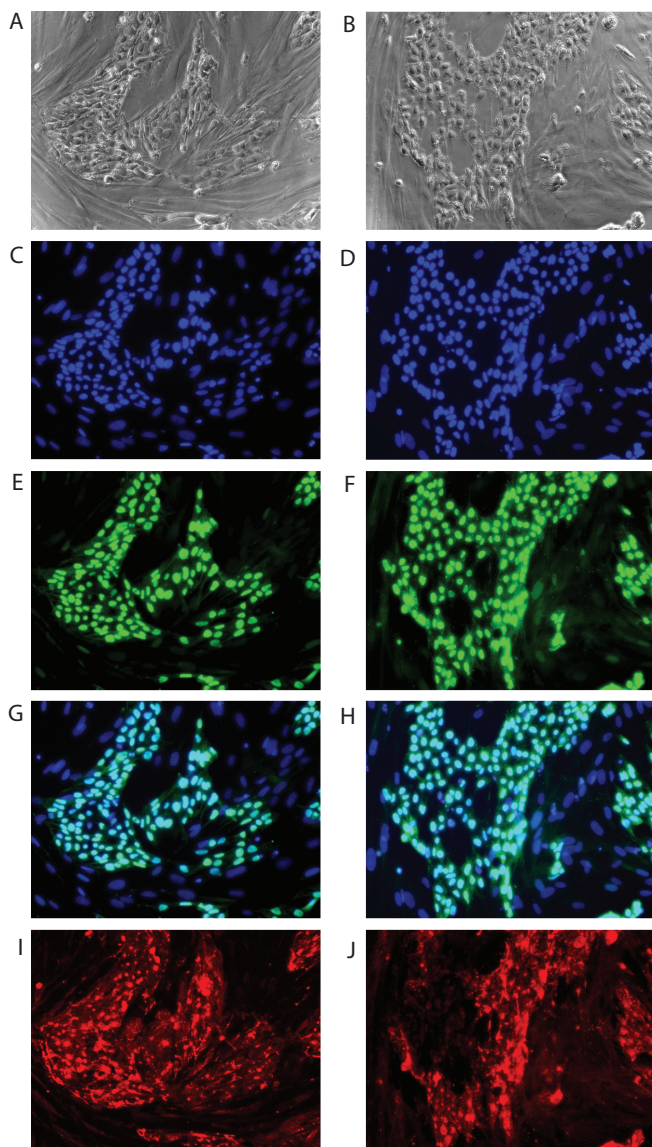


Figure 4. BG01v cells cultured in KnockOut™ SR XenoFree maintain expression of common hESC markers. BG01v cells were cultured on HFF + CELLstart™ substrate for 5 passages prior to immunocytochemical staining. Left panels: KnockOut™ SR control. Right panels: KnockOut™ SR XenoFree. (A, B) Phase contrast image. (C, D) DAPI. (E, F) OCT4. (G, H) DAPI + OCT4. (I, J) SSEA-4.

Maintains pluripotency of hESCs and human iPSCs

Human ESCs and iPSCs proliferate indefinitely while retaining pluripotency. In order to assess whether hESCs were still in an undifferentiated state, immunocytochemistry was performed to determine the presence of cell surface markers such as stage-specific embryonic antigen (SSEA-4) and the common hESC marker/transcription factor OCT4 (Figure 4). Characterization by RT-PCR also confirmed pluripotency (Figure 5).

Like hESCs, human iPSCs have been derived, expanded, and grown in KnockOut™ SR XenoFree (Figure 6). This xeno-free environment will help create models that have a direct path to the clinic.

Maintains pluripotent differentiation capability of hESCs and human iPSCs

Human ESCs and human iPSCs differentiate into cells of the three germ layers: ectoderm, mesoderm, and endoderm.⁵ Differentiation can be studied *in vitro* through the creation of embryoid bodies and their subsequent outgrowth. Cells taken from embryoid body cultures should show immunocytochemical markers for each of the three germ layers. Figure 5 shows that BG01v cells grown in KnockOut™ SR XenoFree medium can differentiate into ectoderm (PAX6), mesoderm (HAND1), and endodermal (AFP) lineages. GAPDH is a housekeeping control gene.

Preserving hESC gene expression profiles

It is important that a new formulation for hESC and human iPSC cultures does not alter the gene expression profile of these cells. To confirm that KnockOut™ SR XenoFree maintains standard gene expression profiles, Illumina BeadArray™ technology compared

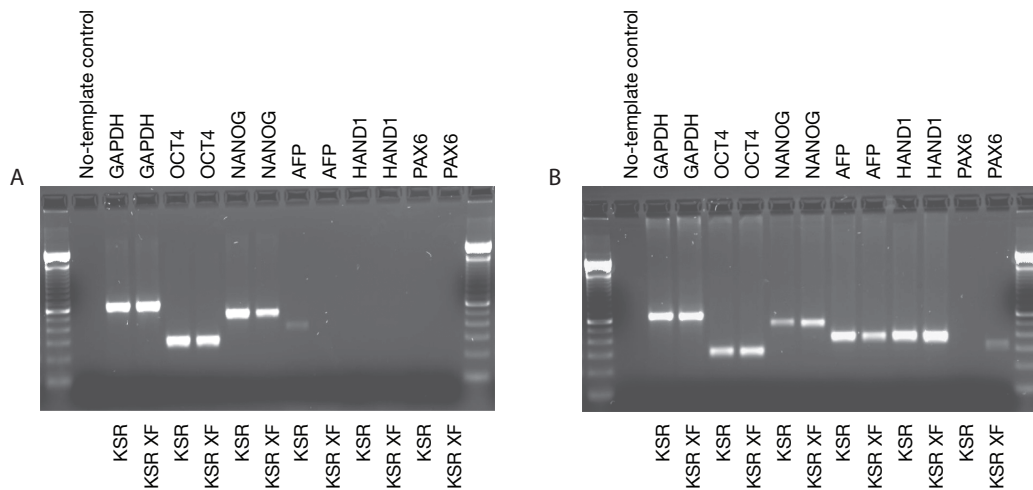


Figure 5 RT-PCR analysis demonstrates that hESCs expanded in KnockOut™ SR XenoFree retain pluripotent differentiation potential. (A) BGO1v cells (passage 10) expanded in either KnockOut™ SR (KSR) or KnockOut™ SR XenoFree (KSR XF) on HFF + CELLstart™ substrate express pluripotency markers NANOG and OCT4. (B) Embryoid bodies generated from BGO1v cells (passage 10) expanded in either KSR or KSR XF on HFF + CELLstart™ substrate express differentiated cell markers alpha-fetoprotein (AFP) (endoderm), HAND1 (mesoderm), or PAX6 (ectoderm). The housekeeping gene GAPDH is shown as a control along with NTC (no-template control).

Cyt49 and BG02 hESC cell lines cultured in KnockOut™ SR Xeno-Free or StemPro® hESC SFM using either Geltrex™ or CELLstart™ substrates (Figure 7). Genes detected with $P < 0.01$ are represented as blue dots along with the correlation (R^2) value. Data points lying outside the red lines indicate genes differentially expressed over 2.5-fold. An R^2 value closer to 1 represents similarity in gene expression patterns.

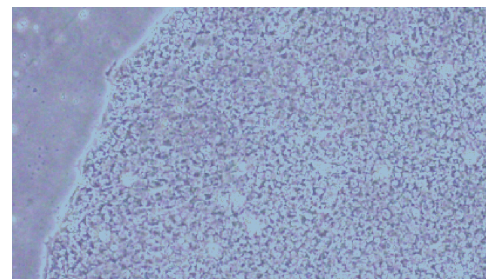


Figure 6 Human iPSCs derived, expanded, and grown in KnockOut™ SR XenoFree without feeder cells using CELLstart™ xeno-free substrate and KnockOut™ SR GF Cocktail. Human iPSCs derived and grown to passage 5. Data provided by Dr. Hidenori Akutsu, National Center for Child Health and Development, Tokyo.

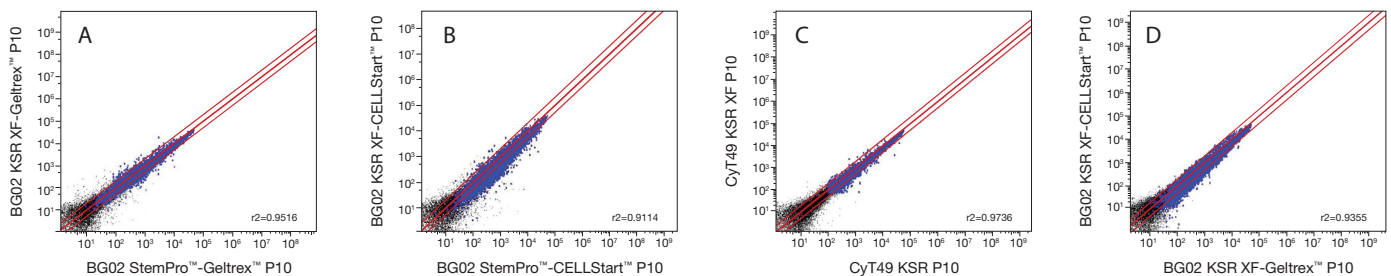


Figure 7 KnockOut™ SR XenoFree maintains global mRNA expression of hESCs. Scatterplot analysis showing global gene expression analysis. hESCs grown on different matrices in different media were analyzed for global gene expression using an Illumina Human 46k array. (A) KnockOut™ SR XenoFree vs. StemPro® hESC SFM on Geltrex™ substrate. (B) KnockOut™ SR XenoFree vs. StemPro® hESC SFM on CELLstart™ substrate. (C) Cyt49 cells grown using KnockOut™ SR XenoFree vs. KnockOut™ SR on human serum-coated plates. (D) KnockOut™ SR XenoFree using Geltrex™ vs. CELLstart™ substrates.

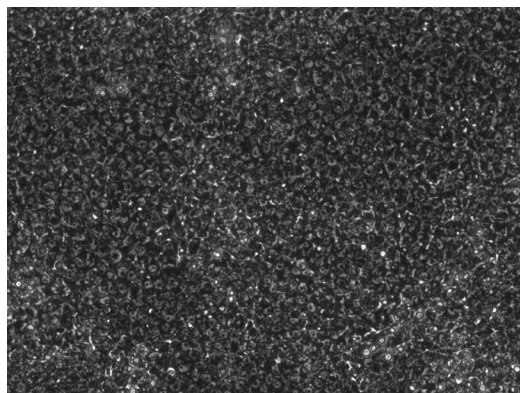


Figure 8. Morphology of hESCs cultured in KnockOut™ SR XenoFree without feeder cells. BG01v cells were cultured using KnockOut™ SR XenoFree Medium containing KnockOut™ SR GF Cocktail directly on CELLstart™ substrate. The phase contrast image shows BG01v at passage 35 in feeder-free conditions. Cells exhibit a high nucleus-to-cytoplasm ratio and have prominent nucleoli.

Can be used feeder-free

KnockOut™ SR XenoFree supports long-term, multi-passive hESC growth in a feeder-free environment when used with KnockOut™ SR GF Cocktail⁴ and CELLstart™ xeno-free substrate. Normal morphology is maintained with no loss in pluripotency (Figures 8 and 9).

References

1. Thomson, J. et al. (1998) Embryonic stem cell lines derived from human blastocyst. *Science* 282:1145–1147.
2. Xu, C. et al. (2001) Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol* 10:971–974.
3. Yu, J. (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318:1917–1920.
4. Wang, L. et al. (2007) Self-renewal of human embryonic stem cells requires insulin-like growth factor-1 receptor and ERBB2 receptor signaling. *Blood* 110: 4111–4119.
5. Pal, R. et al. (2007) A panel of tests to standardize the characterization of human embryonic stem cells. *Regen Med* 2:179–192.

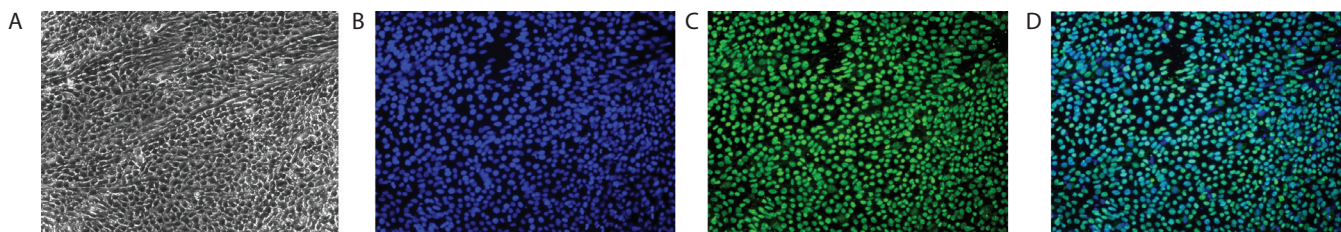


Figure 9. Feeder-free, xeno-free hESC growth. BG01v cells were cultured in KnockOut™ SR XenoFree Medium containing KnockOut™ SR GF Cocktail directly on CELLstart™ substrate without HFF or conditioned medium. At passage 15, cells were fixed and stained for OCT4 expression. Results show that the stem cell phenotype was maintained. (A) Phase contrast. (B) DAPI. (C) OCT4. (D) DAPI + OCT4.

Components required for KnockOut™ SR XenoFree Complete Medium using feeders [human foreskin fibroblasts (HFF)]

Please order these components to make up the complete medium.

KnockOut™ SR XenoFree Complete Medium	Stock concentration	Final concentration	For 100 mL
KnockOut™ D-MEM		1X	82.75 mL
KnockOut™ SR XenoFree		15%	15.0 mL
GlutaMAX™-I	200 mM	2 mM	1.0 mL
NEAA	10 mM	0.1 mM	1.0 mL
bFGF	10 µg*	8 ng/mL	80 µL
2-Mercaptoethanol**	55 mM	0.1 mM	182 µL

*Reconstitute to a stock concentration of 10 µg/mL. **Add only enough 2-mercaptoethanol for that day's use.

Components required for KnockOut™ SR XenoFree Complete Medium using a feeder-free system (CELLstart™ defined xeno-free substrate)

Please order these components to make up the complete medium.

KnockOut™ SR XenoFree Complete Medium	Stock concentration	Final concentration	For 100 mL
KnockOut™ D-MEM		1X	82.0 mL
KnockOut™ SR XenoFree		15%	15.0 mL
GlutaMAX™-1	200 mM	2 mM	1.0 mL
KnockOut™ SR GF Cocktail	50X	1X	2.0 mL
bFGF	10 µg*	8 ng/mL	80 µL
2-Mercaptoethanol**	55 mM	0.1 mM	182 µL

*Reconstitute to a stock concentration of 10 µg/mL. **Add only enough 2-mercaptoethanol for that day's use.

Ordering information

Product	Quantity	Cat. no.
KnockOut™ SR XenoFree	100 mL	A10992-01
	500 mL	A10992-02
KnockOut™ D-MEM	500 mL	10829-018
GlutaMAX™-1	100 mL	35050-061
MEM Non-essential Amino Acids Solution 10 mM (100X)	100 mL	11140-050
bFGF	10 µg	13256-029
2-Mercaptoethanol (1,000X)	50 mL	21985-023
Geltrex™ hESC-Qualified Reduced Growth Factor Basement Membrane Matrix	1 mL	A10480-01
	5 mL	A10480-02
CELLstart™ Substrate	2 mL	A10142-01
KnockOut™ SR GF Cocktail	10 mL	A1058001*

* To order this product and receive the protocol for feeder-free hESC/iPSC culture using the KnockOut™ SR GF Cocktail, contact our Technical Support team at techsupport@invitrogen.com or 800 955 6288.

Keep your workflow xeno-free

CELLstart™ Defined, Xeno-Free
Substrate for Cell Culture



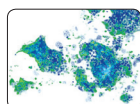
CELLstart™ Substrate ensures hESC and human iPSC attachment when growing in a serum-free medium such as KnockOut™ SR XenoFree. Learn more at www.invitrogen.com/stemcell/cellstart.

StemPro® EZPassage™ Disposable
Stem Cell Passaging Tool



The StemPro® EZPassage™ Tool cuts hESC and human iPSC colonies into uniform pieces for optimal passaging. Learn more at www.invitrogen.com/stempro/ezpassage.

TryPLE™ Select Cell Dissociation Enzyme



Gentle on hESCs and iPSCs, and free of animal-derived components. Learn more at www.invitrogen.com/tryple.

Learn how KnockOut™ SR XenoFree can clear your stem cells' path to the clinic at www.invitrogen.com/stemcell/ksrxf.



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