

# FibroGRO™ Xeno-Free Human Foreskin Fibroblasts

Product Type

Cat. # SCC058

Lot #

pack size: 1 EA

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY



## Certificate of Analysis

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### Description:

Millipore's FibroGRO™ Xeno-Free Human Foreskin Fibroblasts are derived from normal human foreskin and have been isolated and manufactured under xeno-free conditions. Cells are supplied at a low passage number (p3 – p5) and proliferate rapidly when grown in FibroGRO™ LS (low serum) Complete Medium (Cat. No. SCMF002). Rapid proliferation of human foreskin fibroblasts enable efficient reprogramming of the cells to induced pluripotent stem (iPS) cells. Using the lentivirus and protocol detailed in Millipore's STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (Cat. No. SCR530), reprogramming efficiency of 0.1% can be obtained with passage 6 FibroGRO™ Xeno-Free Human Foreskin Fibroblasts. Cells have been validated for reprogramming capacity and tested negative for mycoplasma. Human foreskin fibroblasts provide a useful cell line to aid in the dissection of the mechanism of reprogramming along with being a useful control to ascertain the reprogramming efficiencies of different delivery-based systems (i.e. adenovirus, lentivirus, small molecule, protein-based, episomal and minicircle DNA) or for use as a reprogramming control alongside a specific cell type.

**Kit Component:** 1 x 10<sup>6</sup> cells per vial

**Storage and Stability:** Store in Liquid Nitrogen

**Materials Required But Not Supplied :** FibroGRO™ LS Complete Medium (Cat. No. SCMF002)

### Quality Control Testing :

FibroGRO™ Xeno-Free Human Foreskin Fibroblasts (p6) have been tested and validated to generate iPS cells using STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (Cat. No. SCR530).

### References:

Meng, G., Liu, S., Krawetz, R., Chan, M., Chernos, J., and Rancourt, D. E. (2008) A novel method for generating xeno-free human feeder cells for human embryonic stem cell culture. *Stem Cells Dev.* **17(3)**: 413-422.

### Protocol for Thawing FibroGRO™ Xeno-Free Human Foreskin Fibroblasts

1. Do not thaw the cells until the recommended medium and plasticware are on hand.
2. Remove the vial of FibroGRO™ Xeno-Free Human Foreskin Fibroblasts from liquid nitrogen and incubate in a 37°C waterbath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In the laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of FibroGRO™ LS Complete Medium (Cat. No. SCMF002) (pre-warmed to 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock.**
6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful not to introduce any bubbles. **IMPORTANT: Do not vortex the cells.**
7. Centrifuge the tube at 300 x g for 5 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 4-6 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 10 mL of FibroGRO™ LS Complete Medium (Cat. No. SCMF002) (pre-warmed to 37°C).
10. Plate the cell mixture onto a fresh 10-cm tissue culture plate or T75 flask.
11. Incubate the cells at 37°C in a 5% CO<sub>2</sub> humidified incubator.
12. The next day, exchange the medium with fresh FibroGRO™ LS Complete Medium (pre-warmed to 37°C). Exchange with fresh medium every other day thereafter.
13. When cells are approximately 80% confluent, they can be dissociated with Accutase™ and passaged or alternatively frozen for later use.

Please visit [www.millipore.com](http://www.millipore.com) for additional product information, test data and references.

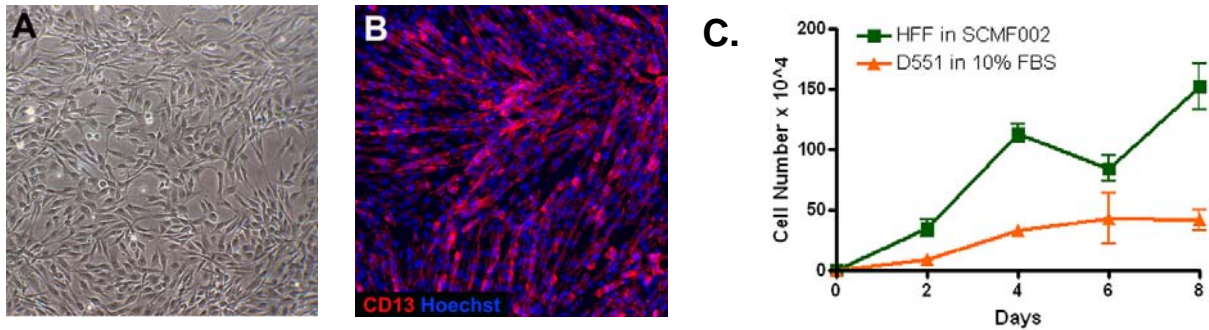
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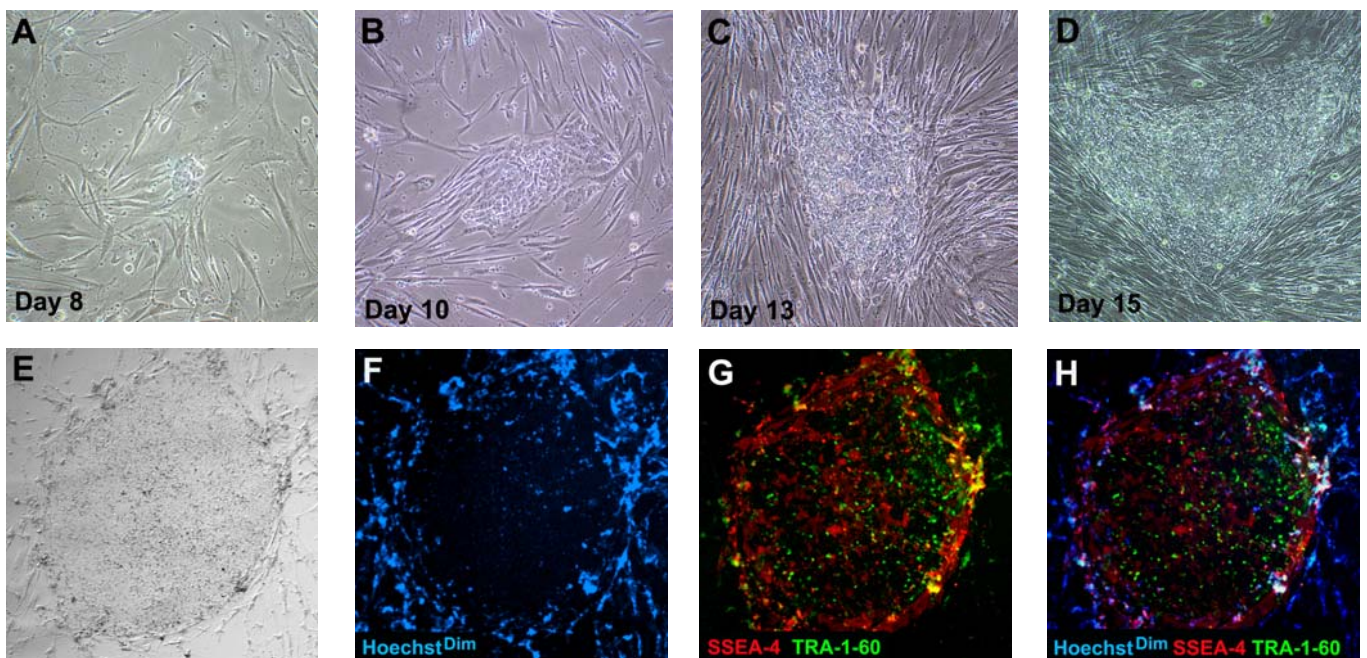
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**Quality Control Testing**



**Figure 1.** FibroGRO™ Xeno-free Human foreskin fibroblasts (Cat. No. SCC058) are grown as monolayers (A), express the fibroblast marker, CD13 PE (B, red) and are negative for pluripotent markers, SSEA-4 and TRA-1-60 (data not shown). Human foreskin fibroblasts cultured in FibroGRO™ LS Complete Medium (Cat. No. SCMF002) proliferate significantly faster than D551 human fibroblasts grown in medium containing 10% FBS (C).

**Timecourse of human iPS colony formation**



**Figure 2.** Timecourse of human iPS colony formation. At day 6, virus-infected human foreskin fibroblasts were dissociated into a single-cell suspension and replated at a density of  $1 \times 10^4$  to  $5 \times 10^4$  cells to each well of a 6-well plate containing irradiated MEFs. Morphology and approximate density of replated cells at Day 8 (A) and Day 10 (B). By Day 13, iPS cell colonies are more visible and different sized iPS cell colonies can be observed ranging in size from ~ 50 cells to several hundred cells (C, D). Colonies that are compact and have human ES-like morphology with defined borders can be selected and manually passaged at around Day 15 –Day 20 (D). By passage 5, fully reprogrammed human iPS cells (E) express human pluripotent markers, TRA-1-60 FITC (G, H, green) and SSEA-4 PE (G, H, red) while downregulating the fibroblast marker, CD13 PE (data not shown). Fully reprogrammed human iPS cells exhibit Hoechst dim phenotype (see colony center in F, H) while non-iPS and differentiated cells exhibited a Hoechst bright phenotype (see the periphery of the colony in F, H), which is surrounded by fibroblast cells and are Hoechst bright). FibroGRO™ Xeno-Free Human Foreskin Fibroblasts (Cat. No. SCC058) were reprogrammed using the STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (Cat. No. SCR530) and live cell staining was performed on passage 5 human iPS cells using the Human iPS Selection Kit (Cat. No. SCR502).

**RELATED PRODUCTS**

cat #	description
SCMF001	FibroGRO™ Complete Medium (serum-free) for Human Fibroblasts
SCMF002	FibroGRO™ LS Complete Medium (low serum) for Human Fibroblasts
SCR510	STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (1 vial)
SCR530	STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (3 vials)
SCR502	Human iPS Selection Kit

antibodies Multiplex products biotools cell culture enzymes kits proteins/peptides siRNA/cDNA products

Please visit [www.millipore.com](http://www.millipore.com) for additional product information, test data and references

28820 Single Oak Drive • Temecula, CA 92590

Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502

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