Rev. 0

Human Dermal Fibroblasts-GFP (HDF-GFP)

Catalog #2310-GFP

Cell Specification

GFP expressing Human Dermal Fibroblasts – GFP (HDF-GFP) are derived from primary human dermal fibroblasts infected with a recombinant, replication-incompetent lentivirus encoding the Green Fluorescent Protein (GFP) [1]. The GFP gene is stably integrated into the host cell genome, ensuring consistent, robust, and long-term expression of green fluorescence across multiple passages without the need for continuous antibiotic selection.

Fibroblasts have been extensively used for a wide range of cellular and molecular studies as they are one of easiest types of cells to grow in culture. Dermal fibroblasts switch from a proliferative, migratory phase to a contractile, matrix-remodeling phase during wound healing [2]. In addition, they secrete large quantities of hyaluronan in response to inflammatory stimuli [3]. These cells retain the characteristic morphology, durable growth kinetics, and matrix-secreting properties of parental dermal fibroblasts, while providing a robust fluorescent signal for advanced imaging and tracking.

HDF-GFP from ScienCell Research Laboratories is isolated from neonatal human skin, and cryopreserved after being transduced, and delivered frozen. Each vial contains > 5 x 10^5 cells in 1 ml volume. HDF-GFP are characterized by their spindle morphology and immunofluorescence with an antibody specific to fibronectin, while maintaining GFP expression during culture. HDF-GFP are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HDF-GFP are guaranteed to further expand for 15 population doublings and maintain a >85% GFP ratio under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Fibroblast Medium (FM, Cat. #2301) for culturing HDF-GFP *in vitro*.

Product Use

HDF-GFP are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Ghaleh, H. E. G., Bolandian, M., Dorostkar, R., Jafari, A., & Pour, M. F. (2020). Concise review on optimized methods in production and transduction of lentiviral vectors in order to facilitate immunotherapy and gene therapy. *Biomedicine & Pharmacotherapy*, *128*, 110276.

[2] Kisiel, M. A., & Klar, A. S. (2019). Isolation and culture of human dermal fibroblasts. In *Skin tissue engineering: methods and protocols* (pp. 71-78). New York, NY: Springer New York.

[3] Stair S, Carlson KW, Shuster S, Wei ET, Stern R. (2002) "Mystixin peptides reduce hyaluronan deposition and edema formation." Eur J Pharmacol. 450: 291-6.

Rev. 0

Instructions for culturing primary cells

Caution:

Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Minimize the exposure of cells to ambient light and excitation light during microscopy to prevent photobleaching and phototoxicity. Please note that confluent primary fibroblasts or over-matured extracellular matrix may exhibit mild background autofluorescence. Adjust threshold settings appropriately using non-transduced HDFs as a negative control if necessary.

Special Notification:

Always check the GFP signal before passaging or expanding the cells under a standard inverted fluorescence microscope. Healthy HDF-GFP cells should exhibit bright, uniform green fluorescence localized throughout the cytoplasm and process extensions. The GFP-positive population should remain >85%. If a decline in the percentage of GFP-positive cells is observed or suspected, we recommend incorporating 0.5–2 µg/mL of Puromycin (Cat. #0553) into the culture medium as needed to restore population purity.

Initiating the culture:

Note: ***ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.***

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). To obtain a 2 µg/cm^2 poly-L-lysine-coated culture vessel, add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37ºC incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, fibronectin-coated culture vessel.

   Note: Dilution and centrifugation of cells after thawing are not recommended as these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in fibronectin-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.

8. Do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Approximately 24 hours post-thawing, observe the cells under a fluorescence microscope to verify initial GFP expression and cell attachment.
3. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
4. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90%-95% confluency.
2. Prepare poly-$_L$-lysine-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, 0.05% (T/E, Cat. #0183), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 5 ml DPBS and 5 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

   Note: We recommend using ScienCell 0.05% T/E solution which is optimized to minimize cell damage due to over trypsinization. If 0.25% T/E solution (Cat. #0103) is used, then 9 ml of DPBS and 1 ml of 0.25% T/E solution should be used.

   Caution: Do NOT use undiluted trypsin when subculturing primary cells.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Once the cells completely round up, transfer T/E solution from the flask to a 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another minute (no solution in the flask at this time).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Gently resuspend cells in culture medium.
12. Count and plate cells in a new poly-$_L$-lysine-coated culture vessel with the recommended cell density. A seeding density of 5,000 cells/cm^2 is recommended.

Note: We do not recommend cryopreservation of cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.