
Product description

Name: Immortalized Fibroblastic Reticular cell line

Parental cell: Human lymphatic fibroblasts

Organism: Human

Tissue: Lymph Node

Growth properties: Adherent

Model: Immortalized Line

Model description: Human lymphatic fibroblasts were immortalized by transfecting them with a vector that expresses HPV16 E6 and E7 genes. Primary fibroblastic reticular cells (FRCs) are stromal cells found in secondary lymphoid organs, which are major sites for HIV-1 infection of CD4+ T cells. Using the immortalized FRCs it was shown that FRCs capture HIV-1 particles and facilitate infection of T cells when FRCs contact T cells, increasing HIV-1 spread via trans-infection.

CRISPR: No

Production details: Immortalized Human lymphatic fibroblasts were generated by transfection of cells with vector pLXSN16E6E7, which encodes HPV E6 and E7, and subsequent selection in fibroblast growth supplement containing G418. A clone was isolated from G418-resistant cells by limiting dilution and cultured in fibroblast growth medium without G418.

Contributor(s)

Inventor: Akira Ono

Institute: University of Michigan

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Recommended medium: Fibroblast medium (FM) (ScienCell Catalog #2301) + 2% FBS + Pen/Strep + FGS

Additional medium (washing): RPMI-1640 + 10% FBS + Pen/Strep/Glutamine

Cell Density: 80000/ mL or 16000/cm²

Subculture: Split every 2-3 days, maintain for up to two months post-revival of frozen cells

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO₂

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature**. To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension to a centrifuge tube using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells.
6. Wash by adding 15 - 20 mL of medium **dropwise**, while gently swirling the tube.
7. Centrifuge the cell suspension at **1200 rpm for 5 minutes** at room temperature.
8. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
9. Gently add required volume of FM medium and transfer to a suitable cell culture flask. It is recommended to thaw into a T-25 flask to maintain high density. Once confluent, cells can be plated in a T-75 flask.
10. Examine the cultures after 24 hours and split every 3-4 days.
11. To subculture, use 0.25% trypsin + EDTA and incubate for 3-5 min at 37°C. Add prewarmed RPMI medium to wash and transfer the cells to a 15 mL tube. Centrifuge at 1,200 rpm for 5 min, aspirate the supernatant and resuspend the cell pellet in prewarmed FM medium.

Note: The confluence of iFRCs is a very important factor for growth. If cell expansion is not observed, please thaw frozen stock and culture for 3-4 days, then trypsinize and re-plate into the same culture dish/flask to keep cells at a relatively higher confluence. Once cells reach around 100% confluence, continue culture as per standard recommendations. Additionally, when the cells are passaged, re-plating 40-50% of the cells might improve growth.

References

- Murakami et al. 2018. Nat Commun. 22:9(1):2436. PMID: 29934525

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner:
Immortalized Fibroblastic Reticular cell line, was invented by Akira Ono (CancerTools.org #156505).

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

While CancerTools.org has made all reasonable efforts to ensure that the information provided by CancerTools.org and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.