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Product Information

Rat Embryonic Fibroblasts (REF)

Catalog Number	10RA-015	Cell Number	1.0 million cells/vial
Species	*Rattus norvegicus*	Storage Temperature	Liquid Nitrogen

Description

Rat Embryonic Fibroblasts (REF) are used to support the growth of rat and human pluripotent stem cells [1]. REF not only provide a substrate for pluripotent stem cells to grow on, but also secrete critical growth factors to maintain stem cell pluripotency. REFs are isolated from rat embryos and used at early passages [2]. To serve as feeder cells, REF must be treated with mitomycin C or by irradiation to prevent cell proliferation. The treated cells can also be used to generate conditioned medium for feeder-free culture of pluripotent stem cells.

iXCells Biotechnologies provides high quality Rat Embryonic Fibroblasts (REF), which are isolated from embryonic day 14 rat embryos and cryopreserved at P0, with >1 million cells in each vial. REFs express fibronectin and are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. They can further expand for 5 population doublings in Fibroblast Growth Medium (Cat# MD-0011) under the condition suggested by iXCells Biotechnologies..

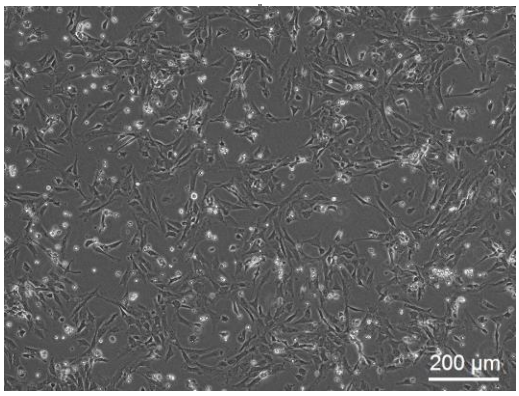

Figure 1. Rat Embryonic Fibroblasts (REF) (phase contrast)

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Product Details

Tissue	Rat Embryonic Fibroblasts (REF)
Package Size	1.0 x 10^6 cells/vial
Passage Number	P0
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Fibroblast Growth Medium (Cat# MD-0011)

Protocols

Thawing of Frozen Cells

1. Upon receipt of the frozen Rat Embryonic Fibroblasts (REF), it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the risk of contamination.
3. Pipette the cells into a 15 mL conical tube with 5ml fresh **Fibroblast Growth Medium (Cat# MD-0011)**.
4. Centrifuge at 1,000 rpm (~220g) for 5 minutes under room temperature.
5. Remove the supernatant and resuspend the cells in fresh culture medium.
6. Culture the cells in 100 mm culture dish or T75 flask. Change the medium every other day until the cells reach 70-80% confluency.

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Standard Culture Procedure

1. Rat Embryonic Fibroblasts (REF) can be cultured in **Fibroblast Growth Medium (Cat# MD-0011)**.
2. When cells reach ~80-90% confluence, remove the medium, and wash once with sterile PBS (5ml/T75 flask).
3. Add ~2.5ml of 0.25% Trypsin-EDTA to the flask and incubate for ~3 minutes at 37°C. Neutralize the enzyme by adding 2-3 volumes of cell culture medium.
4. Centrifuge 1,000rpm (~220g) for 5min and resuspend the cells in desired volume of medium.
5. Seed the cells on the culture vessels at 5 × 10^3 cells/cm^2.

References

[1] Bradley, A. (1987). Production and analysis of chimaeras. In Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, (ed. E. J. Robertson), pp. 113-151. Oxford: IRL Press.

[2] Ping Li, Chang Tong, Ruty Mehrian-Shai, Li Jia, Nancy Wu, Youzhen Yan, Robert E. Maxson, Eric N. Schulze, Houyan Song, Chih-Lin Hsieh, Martin F. Pera, and Qi-Long Ying. (2008) Germline Competent Embryonic Stem Cells Derived from Rat Blastocysts. *Cell*. 135(7): 1299-1310.

Disclaimers