

Description

The irradiated feeder cells, derived from the 3T3-J2 mouse fibroblast cell line from Richard Schlegel's lab at Georgetown University, support the expansion of epithelial cells in CR technology. The feeder cells are irradiated to arrest cell proliferation. The irradiated feeder cells are prepared in ready to use format, the feeder cells do not require plating prior to the seeding of epithelial cells.

The irradiated feeder cells are provided as a cryopreserved vial with 2 – 3 million cells per vial in 0.5 mL. Each vial of irradiated feeder cells is sufficient to seed three T-25 flasks or one T-75 flask.

Intended Use

For Research Use Only.

The irradiated feeder cells can support epithelial cell culture for up to one week. Addition of fresh irradiated feeder cells may be required if the epithelial cells take more than one week to reach confluence. To use the irradiated feeder cells a seeding density of 3.0×10^4 viable cells/cm², with a range of 2.5×10^4 to 4.0×10^4 viable cells/cm², is

recommended to support epithelial cell growth using CRM medium, Propagenix Catalog No. 276-101. Culture medium should be renewed every 3 – 4 days.

Storage Information

1. Vapor phase of liquid nitrogen.

Quality Assurance

1. The irradiated feeder cells have been tested prior to shipment to meet quality control specifications. Each lot is tested for bacterial and fungal contaminations, mycoplasma and interspecies contaminations, cell density, cell viability, fill volume and functionally tested to support the growth of epithelial cells in CR Technology.
2. Additional information is available upon request.

Limitations

The irradiated feeder cells are intended to support the growth of epithelial cells when used together with the CRM medium completed with Cholera toxin, Propagenix Catalog No. 276-101.

Procedure

1. Prior to use, calculate the number of irradiated feeder cells required for the vessels to be seeded.
2. Thaw the number of vials of irradiated feeder cells required by gentle agitation in a 37°C water bath. Thawing should be complete within approximately 2 minutes.
3. As soon as the vial contents are thawed, remove the vial(s) from the water bath and decontaminate by spraying the outside of the vial(s) with 70% ethanol.
4. Transfer the vial contents to a conical centrifuge tube containing 0.5 mL of warm CRM complete medium and mix gently by pipetting. If more than one vial of the irradiated feeder cells is needed,

increase the amount of CRM complete medium accordingly. For example, use 1 mL of CRM complete medium for two vials of irradiate feeder cells and so on.

5. Count the viable irradiated feeder cells per mL using a hemocytometer or automatic cell counter using standard Trypan Blue exclusion.
6. Seed the irradiated feeder cells at 3.0×10^4 viable cells/cm² (recommended range of $2.5 - 4.0 \times 10^4$ viable cells/cm²).
7. Seed desired number of epithelial cells into the flask with the irradiated feeder cells. Bring the total volume of CRM complete medium to 1 mL per cm², for example 5 mL for one T-25 flask. Incubate the epithelial and feeder cell culture in a 37°C incubator with 5% CO₂.
8. Change the growth medium every 3 – 4 days.

Limited Use Label License (LULL)

Conditional Reprogramming (CR) Technology, covered under US Patent No. 9,279,106 (issued March 8, 2016), and subsequent patent applications pending in the US and other Jurisdictions.

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