3T3-J2 Irradiated Feeder Cells

Irradiated 3T3-J2 mouse fibroblasts, frozen

Catalog #100-0353 0.5 mL

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Product Description

3T3-J2 Irradiated Feeder Cells are optimized to support epithelial cell expansion in Conditional Reprogramming (CR) Medium (Catalog #100-0352; Liu X et al. 2012; Liu X et al. 2017; Suprynowicz FA et al.). The cells are mitotically inactivated by radiation, but maintain metabolic activity. 3T3-J2 Irradiated Feeder Cells are cryopreserved and can be used directly for seeding epithelial cells without plating in advance. Each vial contains 2 - 3 x 10^6 cells and can be used to seed up to three T-25 cm^2 flasks or one T-75 cm^2 flask. 3T3-J2 Irradiated Feeder Cells are intended for Research Use Only.

Stability and Storage

Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum viability. Thawed samples must be used immediately.

Precautions

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product do not use sharps such as needles and syringes. STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing (before washing) by our test methods.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Conditional Reprogramming (CR) Medium	100-0352
Cholera toxin	e.g. Sigma C8052
DNase I Solution (1 mg/mL)	07900

Handling / Directions For Use

The following protocol is for thawing 3T3-J2 Irradiated Feeder Cells into complete Conditional Reprogramming (CR) Medium (CR Medium + cholera toxin). For complete instructions on using 3T3-J2 Irradiated Feeder Cells with complete CR Medium to support expansion of epithelial cells, refer to the Product Information Sheet (PIS) for CR Medium (Document #10000007811), available at www.stemcell.com or contact us to request a copy.

IMPORTANT: To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing; step 6). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile technique when processing thawed cells.

1. Prepare complete CR Medium as directed in the applicable PIS. Warm medium in a 37°C water bath.
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
4. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.

   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
5. Wipe the outside of the vial with 70% ethanol or isopropanol.

6. Remove a 20 µL aliquot of cells for counting. If using Trypan Blue (Catalog #07050) to assess viability, for ≥ 1 x 10^6 cells we suggest adding a minimum of 20 µL of medium and recording the volume of medium added. For < 1 x 10^6 cells, dilute directly in 20 µL Trypan Blue. Set diluted aliquot aside until step 13. See Tips section for more details on performing cell counts with a hemocytometer.
7. Transfer the remaining cell suspension to a 15 mL conical tube (e.g. Catalog #38009).
8. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 15 mL tube.
9. Wash by adding 4.5 mL of medium dropwise, while gently swirling the tube.
10. Centrifuge the cell suspension at 300 x *g* for 10 minutes at room temperature (15 - 25°C).
11. After centrifugation, remove and discard the supernatant without disturbing the cell pellet. Add 1 mL of medium to resuspend the cell pellet.
12. If cells are starting to clump, add 100 µg DNase I Solution (1 mg/mL; Catalog #07900) per mL of cell suspension and incubate at room temperature for 15 minutes.

    NOTE: Do not add DNase I Solution if the cells will be used for DNA or RNA extraction.
13. Perform a cell count on the diluted aliquot from step 6.
    NOTE: Cell loss can be expected during the wash step. Performing a cell count after washing is recommended.
14. Cells are now ready for use in downstream applications.

Tips

For a protocol on performing total nucleated cell counts using a hemocytometer, refer to https://www.stemcell.com/techtips.

Related Products

For a complete list of related products available from STEMCELL Technologies, including specialized cell culture and storage media, antibodies, cytokines, and small molecules, visit www.stemcell.com or contact us at techsupport@stemcell.com.

References

Liu X et al. (2017) Conditional reprogramming and long-term expansion of normal and tumor cells from human biospecimens. Nat Protoc 12(2): 439–51.

Liu X et al. (2012) ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol 180(2): 599–607.

Suprynowicz FA et al. (2012) Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells. Proc Natl Acad Sci U S A 109(49): 20035–40.

3T3-J2 Irradiated Feeder Cells are manufactured by Propagenix Inc.