

Conditional Reprogramming (CR) Medium

Conditional reprogramming medium for epithelial cell expansion

Catalog #100-0352

250 mL



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Product Description

Conditional Reprogramming (CR) Medium is optimized to use with 3T3-J2 Irradiated Feeder Cells (Catalog #100-0353) to expand epithelial cells without accumulating karyotypic modifications. Epithelial cells seeded into CR Medium revert to a stem-like state that promotes long-term expansion, while maintaining differentiation potential (Liu X et al. 2012; Liu X et al. 2017). This conditional proliferative capability is deactivated once the medium is removed from the cells (Liu X et al. 2012; Liu X et al. 2017). CR Medium is suitable to propagate primary epithelial cells from healthy or tumor tissues (airway, retina, prostate, breast, intestine, pancreas, liver biliary duct, etc.; Liu X et al. 2012; Liu X et al. 2017; Suprynowicz FA et al.) from multiple species, including humans, mouse, and rat. This product is free of antibiotics and intended for research use only.

NOTE: CR Medium must be supplemented with cholera toxin prior to use.

Properties

Storage: Store at -20°C.

Shelf Life: Stable for 3 years from date of manufacture (MFG) on label. Protect from prolonged exposure to light.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Cholera toxin	e.g. Sigma C8052
3T3-J2 Irradiated Feeder Cells	100-0353

Preparation of Complete CR Medium

Use sterile technique to prepare complete CR Medium (CR Medium + cholera toxin). The following example is for preparing 250 mL of complete CR Medium. If preparing other volumes, adjust accordingly.

1. Thaw CR Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 1 month.

2. Prepare cholera toxin stock solution by reconstituting cholera toxin in CR Medium to a final concentration of 1 mg/mL. Mix thoroughly.

NOTE: If not using immediately, aliquot and store at 2 - 8°C for up to 6 months.

3. Add the appropriate volume of cholera toxin stock solution (1 mg/mL; prepared in step 2) to 250 mL of CR Medium to achieve a final concentration of 8.6 - 20 ng/mL.

For example, add 2.2 µL of cholera toxin stock solution (1 mg/mL) for a final concentration of 8.6 ng/mL.

Mix thoroughly.

NOTE: Complete CR Medium does not contain antibiotics; add antibiotics if desired.

NOTE: If not using immediately, store complete CR Medium at 2 - 8°C for up to 1 month. Do not exceed the shelf life of the individual components.

Directions for Use

CO-CULTURE OF EPITHELIAL CELLS WITH 3T3-J2 IRRADIATED FEEDER CELLS

The following protocol is for expanding cultured primary epithelial cells in a single T-25 cm² flask using 3T3-J2 Irradiated Feeder Cells and complete CR Medium. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: In this protocol, 3T3-J2 Irradiated Feeder Cells and epithelial cells are seeded at the same time.

1. Prepare complete CR Medium (see Preparation section). Warm in a 37°C water bath.
2. Thaw and prepare 3T3-J2 Irradiated Feeder Cells. For complete instructions, refer to the applicable Product Information Sheet (Document #10000007812), available at www.stemcell.com, or contact us to request a copy.
3. Add 7.5×10^5 freshly thawed and washed 3T3-J2 Irradiated Feeder Cells to 5 mL of complete CR Medium. This number of feeder cells will result in a final seeding density of 3×10^4 viable cells/cm² (a range of $2.5 - 4 \times 10^4$ viable cells/cm² is recommended).
4. Add the desired number of epithelial cells to the feeder cell suspension.
5. Add the cell suspension to the T-25 cm² flask and incubate at 37°C and 5% CO₂. Perform a full-medium change every 2 - 3 days.

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 USA* 109(49): 20035–40.

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