

Conditioned Medium



*From Irradiated 3T3-J2 Mouse Fibroblasts for Epithelial Cells.
Catalog # 256-100*

Description

Propagenix Conditioned Medium is used for in vitro expansion of primary non-keratinocyte epithelial cells derived from either normal or diseased tissues. It is generated from irradiated 3T3-J2 mouse fibroblast cells (Propagenix Catalog No. PF-1100) and CRM medium (Propagenix Catalog No. 276-101). The 3T3-J2 material used by Propagenix is derived using a clone obtained under an exclusive license granted to Propagenix for intellectual property created at Georgetown University in the laboratory of Professor Richard Schlegel. The Conditioned Medium contains nutrients, fetal bovine serum and growth factors required to support cell growth in the absence of growth-arrested feeder cells when supplemented with Cholera toxin. Cholera toxin should be purchased separately to supplement the medium.

CM does not contain antibiotics.

Intended Use

For Research Use Only.

Storage Information

1. Store Conditioned Medium at -20°C until required.
2. When ready to use, thaw Conditioned Medium overnight at 2° to 8°C protected from light.
3. Once thawed, medium is stable for up to two weeks at 2° to 8°C.
4. If you think it is unlikely you will use the entire contents of the bottle within two weeks, we recommend aliquoting the medium into smaller containers and re-freezing one additional time

before loss of activity is observed, storing new aliquots at -20°C. Prepare aliquots based upon anticipated use within 2 weeks.

Supplement Medium

Prior to use, the Conditioned Medium must be completed with Cholera toxin. Sigma Catalog No. C8052 and Enzo Life Sciences Catalog No. BML-G117-0001 are recommended commercial sources for Cholera toxin.

1. Aseptically rehydrate Cholera toxin in sterile distilled water or CRM to a final stock concentration of 1 mg/mL.
 - a) For example, rehydrate 1 mg Cholera toxin in 1 mL CRM medium.
2. Aseptically add 1 mg/mL Cholera toxin stock solution to 100 mL of CRM medium to a final concentration of 8.6 ng/mL.
 - a) To make 8.6 ng/mL final concentration, add 0.9 µL Cholera toxin stock solution prepared in step 1.
3. The stock solution of Cholera toxin should be aliquoted and stored at 2° to 8°C for up to six months.

Quality Assurance

1. Conditioned Medium is tested prior to shipment to meet quality control specifications. Each lot is tested for sterility, pH, visually inspected for appearance, and functionally tested to support the growth of epithelial cells.
2. Additional information is available upon request.

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Usage Protocol

1. Transfer desired amount of Conditioned Medium (CM) to a vented T-flask and place at 37°C in a 5% CO₂ humidified incubator for 1 – 3 hours to allow the CM pH to equilibrate. Typically, 0.2mL CM per cm² is adequate, e.g., use 5 mL of CM for a 25 cm² flask.
2. Change the Conditioned Medium every 2 – 3 days until the cells approach 80% - 90% confluence. We recommend that you do not let the cells become fully confluent, it is likely to result in reduced expansion potential of the cultured cells. NOTE: Typically, in our hands most epithelial cells grow at a slower rate in Conditioned Medium than they do compared with their growth rate using Conditional Reprogramming Medium (Propagenix Catalog No. 276-101) in co-culture with growth-arrested 3T3-J2 feeder cells (Propagenix Catalog No. PF-1100). This is normal and to be expected.
3. Return remaining CM to the recommended storage conditions for up to two weeks at 2-8°C.

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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