

CRL-3541[™]

Description

MPC 712LT2 is a pheochromocytoma developed from heterozygous Nf1 knockout mice. MPC 712LT2 offers an alternative model to PC12 cells (ATCC CRL-1721) for the study of genes and signaling pathways that regaulate cell growth and differentiation in adrenal medullary neoplasms and are a unique model for studying the regulation of PNMT expression.

Organism: Mus musculus, mouse Cell Type: neuroendocrine cell Tissue: Adrenal gland; Medulla

Morphology: Rounded

Growth properties: Aggregate **Disease:** Pheochromocytoma **Cells per vial:** ≥ 1.0 x 10⁶

Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL₂

ATCC determines the biosafety level of a material based on our risk assessment as

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guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*), U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C

Atmosphere: 95% Air, 5% CO₂

Handling Procedures

Complete medium:



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The base medium for this cell line is ATCC-formulated RPMI-1640 Medium (RPMI-1640; ATCC 30-2001). To make the complete growth medium, add the following components to the base medium:

- Heat-inactivated horse serum to a final concentration of 10%
- HITES medium supplemented with 5% fetal bovine serum

HITES Medium Formulation:

- 1. 0.005 mg/ml Insulin
- 2. 0.01 mg/ml Transferrin
- 3. 30nM Sodium selenite (final conc.)
- 4. 10 nM Hydrocortisone (final conc.)
- 5. 10 nM beta-estradiol (final conc.)
- 6. extra 2mM L-glutamine (for final conc. of 4.5 mM)
- 7. Fetal bovine serum (FBS; ATCC 30-2020) to a final concentration of 5%

Handling Procedure:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70° C. Storage at -70°C will result in loss of viability.

- 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
- 2. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- 3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 200 to 400x g for 8 to 12 minutes.
- 4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
- 5. Incubate the culture at 37° C in a suitable incubator. A 5% $\rm CO_2$ in air atmosphere is recommended if using the medium described on this product

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sheet.

6. CRL-3541 is highly adherent. If cells are allowed to attach to the plate, they will cease to proliferate. Therefore, for cultivation use either: Corning® Ultra-Low Attachment vessels (VWR catalog # 76441-540, 89089-878, and 89089-960) Vessels coated with Anti-Adherence Rinsing Solution (Stem Cell Technologies catalog # 07010)

Subculturing procedure:

CRL-3541 is highly adherent. If cells are allowed to attach to the plate, they will cease to proliferate. Therefore, for cultivation use either: Corning® Ultra-Low Attachment vessels (VWR catalog # 76441-540, 89089-878, and 89089-960) or Vessels coated with Anti-Adherence Rinsing Solution (Stem Cell Technologies catalog # 07010) Maintain the flasks upright (on the short edge) to improve recovery and proliferation. Perform full fluid change at least once every 10 days, or when culture becomes too acidic. Upon full fluid change, mechanically dissociate the aggregates using the following method: o Collect aggregates and pellet by centrifuging 150 to 400 xg for 8 to 12 minutes. o Remove spent media and resuspend pellet with fresh complete culture media using a 10 mL serological pipette. o Gently triturate the cells the cells using a 10 mL pipette until large aggregates appear dissociated. o Seed as appropriate in the final desired volume. Note: Large "rafts" of aggregates may occur within 2 to 3 days after dissociation. Rafts can be dispersed via gentle pipetting. It is ideal to use a 25 mL pipette(or similar large bore pipette) for this procedure where applicable Note: Aggregates may also be enzymatically dissociated using 0.25% tryspin-EDTA(ATCC catalog # 30-2101) and 80 g/mL Dnase I(Sigma catalog # D-4513). After centrifugation, resuspend the pellet in 2-5 mL of the trypsin-Dnase solution. Gently pipette using a small bore pipette until small agregates are achieved. Add an equal amount of culture medium and centrifuge at 200 x g for 3 to 4 minutes. Resuspend in an appropriate amount of culture medium.

Add appropriate aliquots of the cell suspension to new culture vessels. Do not overdilute aggregates, do not add more than 50% of original cell culture volume during fluid adds or fluid changes.

Reagents for cryopreservation: Gibco™ Recovery™ Cell Culture Freezing Media (ThermoFisher Scientific catalog # 12648-010)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: MPC 712LT2 (ATCC CRL-3541)

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References

References and other information relating to this material are available at www.atcc.org.

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