MPC 4/30 PRR

CRL-3538™

Description

MPC 4/30 PRR is a pheochromocytoma developed from heterozygous Nf1 knockout mice. MCP 4/30 PRR offers an alternative model to PC12 cells (ATCC CRL-1721) for the study of genes and signaling pathways that regulate 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: Epithelial like and/or rounded
Growth properties: Adherent
Disease: Pheochromocytoma
Cells per vial: ≥ 1.0 x 10^6

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.

BSL 2

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

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% CO_2

Handling Procedures

Complete medium:

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% CO_2 in air atmosphere is recommended if using the medium described on this product

sheet.

6. It is recommended to subculture at or before 80% confluence. Cells may not reach 100% confluence and grow in clumps which will become necrotic if not passaged.

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Subculturing procedure: "

It is recommended to subculture at or before 80% confluence. Cells may not reach 100% confluence and grow in clumps which will become necrotic if not passaged. Cells will produce a suspension population that can be discarded at every passage or fluid change.

Before beginning subculture procedure prepare a solution of 0.25% Gibco™ Trypsin (ThermoFisher Scientific catalog # 15050- 065) + 80 µg/mL Deoxyribonuclease I. Deoxyribonuclease I (DNase I) should be added to an aliquot of 0.25% Gibco™ Trypsin fresh prior to use.

To prepare 10 mg/mL DNase I stock solution, aseptically combine: o 100 mg Roche™ DNase I (Sigma catalog # 11284932001) o 10 mL Molecular Grade Water (ATCC catalog # 60-2450) Add 8 µL stock DNase I per 1 mL 0.25% Trypsin immediately prior to use.

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of 0.25% Gibco™ Trypsin (ThermoFisher Scientific catalog # 15050-065) + 80 µg/mL Deoxyribonuclease I) to flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Centrifuge the cell suspension at approximately 150 to 400 x *g* for 8 to 12 minutes to remove dissociation agent.
6. Resuspend the cell pellet in an appropriate amount of complete culture medium
7. If cell clumps are present then clumps should be disrupted using a 5 mL serological pipette followed by a P1000.
8. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 1.0 x 10^5 and 2.0 x 10^5 viable cells/cm^2.
9. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 6.0 X 10^4 and 1.0 X 10^5 cell/cm^2.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: Every 2 to 3 days
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 4/30 PRR (ATCC CRL-3538)

References

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

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