

SOP: Propagation of Renal Cell Carcinoma (RCC) RCC_7860

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Ordering Information

RCC_7860 cells can be ordered from ATCC as a frozen ampule (Cat# CRL-1932).

Name: 786-0, Renal Cell Adenocarcinoma

Sex: Male

Notes:

This is an adherent cell line derived from a primary renal cell adenocarcinoma. The cells display both microvilli and desmosomes, and can be grown in soft agar. The cells produce a PTH like peptides that is identical to peptides produced by breast and lung tumors. The peptide has an N terminal sequence similar to PTH, has PTH like activity, and has a molecular weight of 6000 daltons. Cytogenetic analysis indicates that the cells are hypertriploid.

Materials List

1. DMEM, High Glucose, Pyruvate (Cat# 11995 Gibco)
2. Fetal Bovine Serum (Cat#100-106, Gemini Bio-Products)
3. Non-Essential Amino Acids (Cat#11140 Gibco)
4. L-glutamine (Cat#25030 Gibco)
5. 0.5% Trypsin/0.1%EDTA (Cat# 25300 Gibco)
6. 10cm culture plates
7. Graduated pipets (1, 5, 25mL)
8. Hemocytometer
9. Microscope

Growth Medium for 786-0

DMEM, High Glucose, Pyruvate
10% Fetal Bovine Serum
1% Non-Essential Amino Acids
1% L-glutamine

Procedure

A. Receipt of frozen cells and starting cell cultures.

- 1) Immediately place frozen cells in liquid nitrogen storage incubator.
- 2) Quickly thaw ampoule in 37⁰C water bath
- 3) Transfer thawed cells to a 10cm plate with 10mL of warm growth media.
- 4) Allow cells to recover over night in 37⁰C, 5% CO₂ humidified incubator.
- 5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

- 1) Propagate cells until density reaches 80-90% confluence.
- 2) Decant medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 2 ml of Trypsin/EDTA and return to incubator for 5 minutes.
- 5) Add 6 ml of fresh medium and resuspend cells by gently pipetting.
- 6) Perform 1:3 to 1:10 cell split as needed.
- 7) Record each subculture event as a passage.

C. Maintenance

- 1) Change media the day after seeding and 1-2 times per week thereafter.

Use ~10 mLs of medium per 10cm plate.

D. Harvest

- 1) Remove cells from plates according to protocol described above under 'subculturing'
- 2) Examine viability using trypan blue staining (SOP)

E. Passaging

- 1) Recommend no more than 25 – 30 passages.