

## **SOP: Propagation of Malignant Rhabdoid Tumor (MRT) MRT\_TTC549**

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**Modified by: B. Weissman**

### **Ordering Information**

TTC549 can be ordered from Dr. Timothy Triche, Childrens Hospital of Los Angeles, as a frozen ampule.

Name: TTC549, Malignant Rhabdoid Tumor

#### **Notes:**

This is an adherent cell line derived from a hepatic rhabdoid tumor. Hepatic rhabdoid tumors are extremely rare. This cell line has a large deletion on 22q, which indicates numerous genes in addition to SNF5 are deleted.

### **Materials List**

1. RPMI 1640 (Cat# 11875 Gibco)
2. Fetal Bovine Serum (Cat# 26140 Gibco)
3. 0.5% Trypsin/0.1%EDTA (Cat# 25300 Gibco)
4. T-225 culture flasks
5. Graduated pipets (1, 5, 25mL)
6. Hemocytometer
7. Microscope

### **Growth Medium for TTC549**

RPMI 1640

10% FBS

### **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 37<sup>0</sup>C water bath
- 3) Transfer thawed cells to a T75 flask with 20ml of warm growth media.
- 4) Allow cells to recover over night in 37<sup>0</sup>C, 5% CO<sub>2</sub> 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 70-80% confluence.
- 2) Decant medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 2 ml of Trypsin/EDTA and return to incubator for 5-10 minutes.
- 5) Add 6 ml of fresh medium and resuspend cells by gently pipetting.
- 6) Perform 1:3 to 1:8 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 ~35 mLs of medium per T225 flask.

**D. Harvest**

- 1) Do not use cells that have been passed more than 8 times
- 2) Remove cells from flasks according to protocol described above under 'subculturing'
- 3) Examine viability using trypan blue staining (SOP)