SOP: Propagation of Malignant Rhapdoid Tumor (MRT) MRT_A204.1
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Ordering Information

A204.1 can be ordered from the Bernard Weissman Laboratory (UNC) as a frozen ampule.

Name: A204.1, Malignant Rhabdoid Tumor

Notes:
This is an adherent cell line that was derived from a soft tissue rhabdoid tumor isolated from the upper arm. The original A204 cell line has a tendency to accumulate a tetraploid population when passaged in culture, which is a common feature of many types of pediatric tumor cell lines. A204.1 is a diploid clone of the A204 cell line derived by the Weissman laboratory (Charboneau et al., *J. Cell. Physiol.*, 209:422-427, 2006). The parental cell line, A204, can be ordered from the ATCC (HTB-82).

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 A204.1

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 37^0C water bath
3) Transfer thawed cells to a T75 flask with 20ml of warm growth media.
4) Allow cells to recover over night in 37^0C, 5% CO_2 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)