SOP: **Propagation of WERI-Rb-1**
Date modified: **4/20/2009**
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Ordering Information

WERI-Rb-1 may be ordered from ATCC as a frozen ampoule.

Name: WERI-Rb-1, retinoblastoma
ATCC #: **HTB-169**

Notes:
This cell line grows in suspension.

Materials List

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T75 & T225 culture flasks
4. Graduated pipets (1, 5, 25mL)
5. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope

Growth Media for WERI-Rb-1

RPMI-1640 with 2mM L-glutamine
10% FBS
1x Pen-Strep

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^{o}C water bath
3) Transfer thawed cells to a T75 flask at 2-3 X 10^5 density in warm growth medium.
4) Allow cells to recover over night in 37^{o}C, 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 6-8 X 10^5.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Immediately remove cells and pellet at 500 xg for 3 minutes (4^{o}C)
5) Wash cells 2X with 1X PBS.
6) Gently re-suspend cell pellet in warm medium.
7) Seed at density of 2-3 X 10^5.
8) Record each subculture event as a passage

C. Maintenance

1) Change media the day after seeding and every 3-4 days thereafter.

D. Harvest

1) Do not use cells that have been passed more than 25 times
2) Remove cells from flasks according to protocol described above under 'sub-culturing'
3) Examine viability using trypan blue staining.