SOP: Propagation of K562 (ATCC CCL-243)

Information

Name: K562
ATCC #: CCL-243
Tissue: bone marrow
Product Format: frozen
Morphology: lymphoblast
Culture Properties: suspension
Biosafety Level: 1
Disease: chronic myelogenous leukemia; 53-year-old female

ENCODE Number: HO2074

Materials List

1. RPMI1640 (Life Technologies; Cat# 11875-150)
2 Heat Inactivated Fetal Bovine Serum (Life Technologies; Cat# 10082147)
3. Antibiotic-Antimycolic 100X (Life Technologies; Cat#15240-122)
4. T25, T75, T150, T182 culture flasks
5. Graduated pipets (1, 5, 10, 25, 50mL)
6. Freezing medium (Complete growth medium 95%; DMSO, 5%)
7. DMSO (Fisher; Cat#BP-231-100)
8. Cryovials (Sarstedt; Cat #72-694-006)
9. TC20 cell counter (Bio-Rad)
10. Counting Slides (Bio-Rad; Cat# 145-0011)
11. Microscope

Growth Medium for K562

RPMI1640
10% FBS
Anti-Anti; 5 mL per 500 mL of culture medium

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen freezer storage until ready to culture.
2) When ready to start cell culture, quickly thaw ampoule in a 37^{o}C water bath.
3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then transfer contents of ampoule into a 15 mL centrifuge tube with 9-10 mL of warm growth media.
4) Centrifuge at 1,500 rpm for 3 minutes.
5) Aspirate the medium. Suspend the cells in 10 mL complete growth medium and transfer the cells to a T25 cell culture flask.

6) Incubate at 5% CO_2, 37 ^{0}C, humidified incubator. Keep the flask horizontally.

Note: These cells get into log phase in 5 to 7 days. Start counting the cells at day 3. When the total cell number reaches 0.7 to 0.8 X 10^6/mL, split the culture to about 0.4 X 10^6 per mL fresh medium (you could use larger size flasks). From this point on the cells should double every 24 hours.

7) Record each subculture event as a passage.

B. Maintenance and Generation of Seed Stocks

1) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion. A small portion should be set aside as a seed stock. The cell pellet for the seed stock should be resuspended in freezing medium.
2) Cells in freezing medium are dispensed into cryovials (1-2 million cells per 1mL aliquot) and frozen in a -80^{o}C cryo-freezing container overnight.
3) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

C. Harvest

1) Passage cells until the desired number of cells is reached.
2) Remove cells from flasks as described above under "Sub-culture".
3) Examine viability using Trypan blue staining.

Note: In our lab, we thaw a fresh vial of frozen cells, and grow up the cells for 4 to weeks.