SOP: Propagation of GM12878 (Coriell)

Information

Name: GM12878
Coriell #: GM12878
Tissue: blood
Product Format: T25 tissue culture flask
Morphology: B-lymphocyte
Culture Properties: suspension
Biosafety Level:
Disease: chronic myelogenous leukemia; 53-year-old female

ENCODE Number: HO2073

Materials List

1. RPMI1640 (Life Technologies; Cat# 11875-150)
2 Heat Inactivated Fetal Bovine Serum (Life Technologies; Cat# 10082147)
3. Penicillin-Streptomycin 10,000U/mL (Life Technologies; Cat#15140)
4. T25, T75, T150, T182 culture flasks
5. Graduated pipets (1, 5, 10, 25, 50 mL)
6. Freezing medium (RPMI1640 with 20% FBS and 6% DMSO)
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 GM12878

RPMI1640; 2 mM L-glutamine
15% FBS
Pen-Strep (1X)

Procedure

A. Receipt of Cells and Starting Cell Culture

1) GM12878 is shipped in T25 tissue culture flask that has been filled to capacity with carbon-dioxide-equilibrated medium to provide sufficient nutrients for the extended transport times. Upon receipt, cell culture flask should be incubated unopened overnight at 37 ^{0}C. Lymphoblast cultures should be counted the next day and either split if sufficient growth has occurred or the medium volume decreased to yield a cell density of 2-5 X 10^{5} viable cells/mL. Flasks should be incubated in an upright position with vented or loose caps.
2) Incubate in 5% CO_2, 37 ^{0}C, humidified incubator.

Note: Cell density is critical for lymphoblastoid cells. If they grow to more than 1 X 10^6 cells/mL, they start slowing down. If they are diluted to less than 2 X 10^5, they can start dying off. Cells need to clump to some degree to stay healthy.

7) Record each subculture event as a passage.

B. Maintenance and Generation of Seed Stocks

1) Lymphoblastoid cell lines grow in suspension culture with cells clumped in loose aggregates. These aggregates can be dissociated by gently agitating the culture or by gentle trituration with a pipette.
2) Culture should be seeded at a concentration of no less than 2 X $10^{\wedge 5}$ viable cells/mL. In three to four days the culture is either refed with fresh medium or split again depending upon how fast the particular line grows and the desired number of cells. The plateau level for most cultures is about one million viable cells/mL and is reached three to five days after subculturing. The pH of cultures will be quite acidic at this point, appearing distinctly yellow if phenol red is used as an indicator. Culture left in plateau phase tend to exhibit a decrease in viability accompanied by a lengthening of the doubling time.
3) The volume of medium in the flask can affect the growth of cells as the surface to the air ratio is important in maintaining the proper pH of medium. No more than 20 mL of medium should be used in a T25 flask.
4)) Pool sufficient flasks for freezing a seed stock. Dissociate the cell clump by trituration and count the viable cells.
5) 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.
6) 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.