SOP: **Propagation of Jurkat**
Date modified: **12/14/09**
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Ordering Information

Jurkat can be ordered from ATCC as a frozen ampoule.

Name: Jurkat, Clone E6-1 (Acute T Cell Leukemia Lymphocyte)
ATCC #: TIB-152

Notes:
This cell line grows in suspension and should be maintained at a density between 1x10^5 cells/ml and 3x10^6cells/ml.

Materials List

1. RPMI 1640 with 2mM L-glutamine (Cellgro Cat# 10-040-CM)
2. Characterized Fetal Bovine Serum (HyClone Cat# SH30071)
3. T225 culture flasks
4. Graduated pipets (1, 5, 10, 25, 50mL)
5. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
6. Phosphate Buffered Saline (1X PBS) (prepared from 10X stock Cellgro, Cat# 46-013-CM by dilution with sterile deionized water)
7. Freezing medium (growth medium containing 6% DMSO)
8. DMSO, Hybri-Max (Sigma-Aldrich Cat# D2650)
9. Cryovials (Nunc Cat# 368632)
10. Hemocytometer
11. Micropipet w/ P20 tips
12. Microscope

Growth Medium for Jurkat

RPMI 1640 with 2mM L-glutamine
10% FBS
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells, Starting Cell Culture, and Generation of Seed Stocks

1) Immediately place frozen cells in liquid nitrogen freezer storage until ready to culture.
2) Quickly thaw ampoule in 37^{o}C water bath.
3) Transfer thawed cells to a T25 flask with 10ml of warm growth media.
4) Allow cells to recover overnight in 37^{o}C, 5% CO_2 humidified incubator.
5) The next morning, spin down cells out of diluted shipping medium (which contains DMSO) at 500 X g (4^{o}C) for 5 minutes, aspirating medium from cell pellet.
6) Re-suspend cells in warm fresh medium at a volume to yield a density of 1x10^5 cells/ml.
7) Upon reaching the desired number of cells, cells should be spun down, rinsed with 1X PBS, and resuspended in freezing medium.
8) Cells are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen in a -80^{o}C isopropanol cryo-freezing container overnight.
9) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

B. Sub-culture and Maintenance

1) Take cell counts with a hemocytometer every 48 hours to maintain the culture at a cell density between 1x10^5 and 1x10^6 cells/ml (maximal density is at 3x10^6 cells/ml).
2) Add fresh warm medium when appropriate to maintain cell density and expand culture to desired number of cells. Splitting can be performed by centrifuging cells at 500 X g for 5 minutes (4^{o}C), aspirating spent growth medium, and rinsing cell pellet in sterile 1X PBS. Cells should then be resuspended in fresh growth medium to achieve a density 1x10^5 and 1x10^6 cells/ml.
3) Record each subculture event as a passage.

C. Harvest

1) Passage cells until the desired number of cells is reached.
2) Pellet cells and rinse with 1X PBS as described above in "Sub-culture and Maintenance".
3) Examine viability using Trypan blue staining (SOP TP-7).