# Protocol for Culturing Lymphoblasts

## Growth Medium

RPMI 1640 - ThermoFisher # 11875
15% Fetal Bovine Serum (FBS) – ThermoFisher #10437-028
1% (1X) Penicillin-Streptomycin (optional) - ThermoFisher #15140-122

## General Notes

- Growth medium should always be equilibrated in the incubator at 37°C, 5% $CO_2$ for 30 minutes before use
- Always use flasks with vented caps
- Always incubate the flask in an upright position
- No more than 20 ml should be used in a T25 flask

## Thawing Frozen Cells

Frozen cells will be received in 1 ml aliquots on dry ice. Keep cells frozen until thawed for cell culture or place in liquid nitrogen storage if not starting cultures right away. However, it is recommended that if you are going to store the cells for later use, grow up fresh stocks and freeze multiple ampoules. The cells are cryopreserved in 45% RPMI-1640, 50% FBS, and 5% DMSO (tissue culture grade).

1. Place 10 ml of RPMI-1640, 15% fetal bovine serum (FBS), ± antibiotics in a T-25 flask with a vented cap.

2. Allow the media to equilibrate in a 37°C humidified incubator with 5% $CO_2$ in air for 30 minutes. Flasks should be incubated in an upright position.

3. Thaw each ampoule or vial of frozen cells in a 37°C water bath or a beaker of lukewarm water, one at a time.

4. Quickly clean the outside of the ampoule or vial with 70% ethanol. If the cells are in a glass ampoule, carefully break the ampoule being sure to protect fingers from broken glass.

5. Remove the 1 ml of frozen cells with a sterile pipet and place in a T-25 flask with 10 ml of medium. Clearly label flask to identify each cell line.

6. Place the flask in a 37°C humidified incubator with 5% $CO_2$ in the air. Flasks should be incubated in an upright position.

7. After 24 hours, remove 5 ml of medium from the top without disturbing lymphocytes settled on the bottom of the flask and replace with 5 ml of pre-warmed medium.

8. Proceed with lymphoblast cell culture protocol.

## Lymphoblast Culture

1. Three to four days after thawing, count cells. Cells are non-adherent and grow in clumps. Break up clumps by gentle pipetting.
2. Count cells.
3. Ideally, cell concentration should not exceed 2 x $10^6$ cells/ml.
4. Depending on cell concentration refeed, split or freeze cells.
5. Refeed by adding 5-6 ml of equilibrated growth medium.
6. Seed new cultures at no less than 2 x $10^5$ cells/ml.
7. Cells should be frozen at approximately 5 x $10^6$ cells/ml. Follow cell freezing protocol.

## Freezing lymphoblasts

1. Cells should be frozen at approximately 5 x $10^6$ cells/ml.
2. Transfer appropriate volume of cells to 15 or 50 ml conical tube.
3. Centrifuge at 4°C at 100 x g for 10 minutes.
4. Remove medium and resuspend in appropriate volume of cold freezing medium.
5. Transfer 1 ml cells each into cryovial.
6. Place cryovials in freezing chamber and place chamber in -80°C freezer overnight.
7. Transfer cryovials to liquid nitrogen the next day.