Lymphoblast Culture FAQ

1. How are lymphoblast cultures established?

Lymphoblastoid cell lines obtained from Coriell Biorepositories were established by Epstein - Barr virus transformation of peripheral blood mononuclear cells using phytohemagluttinin as a mitogen. All cells lines are free of bacterial, fungal or mycoplasma contamination.

2. What are the basic culture conditions for lymphoblasts?

Recommended Medium:	RPMI 1640 2mM L-glutamine (or equivalent) 15% fetal bovine serum
Culture Conditions:	T25 tissue culture flask with 10-20 ml medium (standing upright) 37°C under 5% carbon dioxide

3. What should I do when I first receive lymphoblast cultures?

Lymphoblast cultures are shipped in T12.5 tissue culture flasks that have been filled to capacity with carbon dioxide-equilibrated medium to provide sufficient nutrients for extended transport times. Upon receipt, cell culture flasks should be incubated unopened overnight at 37°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 200,000 - 500,000 viable cells/ml. Flasks should be incubated in an upright position with vented caps.

4. How do lymphoblasts grow in culture?

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.

Cultures should be seeded at a concentration of no less than 200,000 viable cells/ml. In three to four days the culture should either be re-fed with fresh medium or split 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 cell/ml and is reached three to five days after sub-culturing.The pH of cultures will be quite acidic at this point, appearing distinctly yellow if phenol red is used as an indicator. Cultures left in plateau phase tend to exhibit a decrease in viability accompanied by a lengthening of the doubling time.

The volume of medium in the flask can affect the growth of cells as the surface to air ratio is important in maintaining the proper pH of the medium. No more than 20 ml of medium should be used in a T25 flask.

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5. What are the common problems in growing lymphoblasts?

Factors that can affect the growth characteristics of a cell line include: temperature, pH, the particular lot of medium or serum, depletion of L-glutamine, mycoplasma contamination and the length of time in continuous culture. To control these factors, medium must be pre-warmed to 37°C before addition to the cells, new lots of serum and medium are prequalified on control cell lines, and medium is replaced at regular intervals depending on rate of growth.

Cell cultures are not usually maintained in continuous culture, but frozen to create "seed stocks" for future use.

The percentage of fetal bovine serum used can depend on the individual cell line and can be varied if growth seems slow. The usual range is 10 to 15 percent. Most lymphoblast cell lines grow well in FBS that has NOT been heat-inactivated. Poorly growing lines can be tried in medium containing 20% heat-inactivated FBS (30 min. at 56^{o}C).

Poorly growing lines should be cultured at somewhat higher cell concentrations (300,000 to 500,000 viable cells/ml). Slowly growing lines can be “half-fed” instead of sub-cultured (allow cells to settle to bottom of flask; remove approximately half volume of spent medium; replace with equal volume of pre-warmed fresh medium).

6. How should lymphoblasts be frozen?

1. Pool sufficient flasks for freezing a seed stock.
2. Dissociate the cell clumps by trituration and count the viable cells. Calculate the total number of viable cells.
3. Centrifuge the culture for 10 minutes at 100 (+/- 20) X g at 4-10°C.
4. Re-suspend the cell pellet in the appropriate volume of cold (4-10°C) freeze medium [RPMI 1640 with 30% FBS and 5% DMSO] to yield approximately five million viable cells/ml.
5. Dispense the cell suspension in 1-ml aliquots into plastic or glass cryovials.
6. Freeze at –1 °C / minute to -80°C (either in microprocessor controlled freezer or passively in an isopropanol bath placed in a -80°C freezer overnight).
7. Store in liquid nitrogen (vapor or liquid phase as appropriate).

Recover cells lines by thawing a vial rapidly in a 37°C water bath. Re-suspend the entire contents of the vial in fresh culture medium. The volume to be used will depend on the number of viable cells in the vial and should be adjusted to give a cell density of not less than 200,000 cells/ml.