

RCR testing of Lentivirus Stocks

SUP-T1 cells grow in suspension and do not adhere to a culture plate. Thaw frozen aliquot and add to 15 ml of culture medium (see below). This should be approximately 3×10^5 cells/ml, maintain in culture between 2×10^5 and 2×10^6 cells/ml.

Setup a culture of SUP-T1 cells at 200,000 cells/ml, 5 ml in T25 flask

Culture medium:

RPMI 1640

2 mM L-glutamine

10 mM HEPES

1 mM Sodium Pyruvate

4.5 g/l glucose

1.5 g/l Sodium Bicarbonate

10% Fetal Bovine Serum

Add 5 million lentivirus particles (MOI 5:1), this should result in a 90% infection rate.

Culture and passage the cells 3 times, approximately 3 weeks. To passage SUP-T1 cells, collect medium/cells into a centrifuge tube, pellet cells, resuspend and sub at 1:6 to 1:10.

After 3 passages setup the cells at 200,000 cells/ml incubate for 48 h, test 200 ml of the medium for p24 protein, it should be negative.