UPON RECEIPT, STORE FROZEN AMPOULES OR CRYOVIALS IN LIQUID NITROGEN VAPOR UNTIL YOU ARE READY TO CULTURE THE CELL LINE. **CORIELL WILL NOT PROVIDE REPLACEMENTS FOR CELL LINES DISTRIBUTED AS FROZEN AMPOULES OR CRYOVIALS**. PLEASE VISIT OUR CATALOG WEBSITE TO VIEW OUR SHIPMENT POLICY:
https://www.coriell.org/ >> Ordering >> Shipment Policy

1.0 Safety Precautions for handling Epstein-Barr Virus (EBV):

1.1 If the ampoule is placed in liquid nitrogen upon receipt, special safety precautions should be followed when removing the ampule from storage. The potential exists for glass ampoules to shatter when undergoing rapid temperature change after storage in liquid nitrogen. Protective gloves and clothing should be used and a face shield must be worn when thawing the ampoule.

1.2 Vials of EBV should be thawed by rapid agitation in a 37C water bath. When thawed, the vial should be wiped with 70% ETOH, or an equivalent disinfectant.

1.2.1 If opening a plastic cryovial, the cap can be unscrewed, and the contents transferred aseptically.

1.2.2 If the vial is glass, the neck should be scored with a file that has been disinfected with 70% ETOH, or equivalent alternative. Apply pressure with the thumbs (protected by a special holder or several layers of sterile gauze) on the side opposite the nick should produce a clean break of the neck of the glass ampoule. Transfer the contents of the ampoule using sterile technique.

2.0 Protocol for Transforming B lymphocytes by EBV (abbreviated):

2.1 Collect 5-10 ml of peripheral human blood in collection tubes containing either acid-citrate dextrose or K-EDTA as an anticoagulant.

2.2 Separate peripheral blood mononuclear cells (PBMC) by centrifugation on a Ficoll gradient.

2.3 Wash PBMC two times with RPMI-1640 with 25mM HEPES, pH 7.4.

2.4 Resuspend PBMC in a volume of medium equal to the starting volume of blood (RPMI 1640 with 20% FBS, 2mM L-glutamine or eqivalent, 2.0 g/L glucose, 2.0 g/L sodium bicarbonate and no antibiotics) in a T25 tissue culture flask.

2.5 Add 1 ml EBV and phytohemaglutinin to a final concentration of 10 µg/ml. Incubate at 37C, 5% CO2.

2.6 Twice weekly, examine flask for a change to an acidic pH and the appearance of "clumps" of cells growing in suspension. Adjust the volume of medium in the flask by removing spent medium and adding more or less fresh medium to maintain a slightly acidic pH. Be sure to let the cells settle to the bottom of the flask before adjusting the volume of the medium. By 21 to 35 days in culture, the volume should have increased to approximately 20 mls. Cells should be growing in loose aggregates that can be broken apart by gentle trituration. At this time the lymphocytes can be subcultured at a seeding density of not less than $2x10^5$ viable cells per ml.

2.7 When cell density reaches $8x10^5$ to $1x10^6$ cells per ml, the culture should be split at not less than $2x10^5$ cells per ml or cell stocks should be cryopreserved.