Epstein-Barr Virus Transformation of Lymphoblasts

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This protocol was adapted from "Isolation and Transformation of Lymphocytes," Chapter 7, in Cells (eds. Spector et al.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 1998. This three-volume set is now out of print; however, some of the microscopy methods were republished in Basic Methods in Microscopy, by David L. Spector and Robert D. Goldman.

INTRODUCTION

This protocol describes a method for the transformation of lymphoblasts by Epstein-Barr Virus (EBV). Cells may be isolated from whole blood or taken from cryopreserved, non-immortalized stocks (please see Cryopreservation of Mammalian Culture Cells: Preparation and Recovery of Samples). The source of infectious EBV is the conditioned medium from marmoset cell line B95-8.

MATERIALS

Reagents

Cyclosporin A (Sigma)

Whole blood

EBV-containing supernatant

EBV-containing supernatant is derived from the conditioned medium of a marmoset cell line, B95-8 (available from ATCC). The B95-8 conditioned medium containing EBV should be filtered (22-µm sterilizing filter [Corning]) prior to use to eliminate contaminating marmoset cells.

The B95-8 marmoset cell line produces high titers of infectious EBV. It should be handled in a P2 facility and treated as a biohazardous agent.

Histopaque 1077 (Sigma)

RPMI 1640 Growth Medium

Wall and colleagues recommend the use of conditioned, filtered medium from an EBV-transformed human lymphoblastoid cell line in combination with fresh RPMI 1640. In their hands, the frequency and speed of transformation and cell growth to first passage was enhanced by utilizing 1 part fresh medium:1 part conditioned medium for the initial culture of separated lymphocytes.

Equipment:
Tissue culture flask, 25 cm²
Incubator, 5% CO₂, preset to 37°C
Centrifuge tubes, 15-ml

METHOD

1. Combine 10 ml of whole blood with 10 ml of RPMI 1640 growth medium.

2. Separate lymphocytes by layering 4 ml of diluted blood over 3 ml of Histopaque 1077 in 15-ml sterile centrifuge tubes.

3. Centrifuge the Histopaque column at 300g at room temperature for 30 minutes. Discard the clear plasma layer at the top.

4. Collect the opaque lymphocyte layer and pool the cell suspension (~10 ml) into a 50-ml sterile centrifuge tube.

5. Wash twice in 20 ml of RPMI 1640 growth medium. Pellet at 300g at room temperature for 10 minutes after washes.

6. Prepare the cells for immortalization and growth by resuspending the cell pellet derived from 10 ml of blood in 1 ml of EBV-containing supernatant.

7. Place the cell suspension (1 ml) in a 25-cm² tissue culture flask and set the flask on end (vertically) in a 5% CO₂ atmosphere at 37°C for 1-3 hours.

8. After incubation, add 3 ml of RPMI 1640 growth medium with 0.2 µg/ml of cyclosporin A to the flask and position the flask horizontally so that the cells flow over the normal growth surface.

   *Cultures should be observed every few days for increased cell growth. The formation of cell aggregates is an indicator of immortalization.*

9. Add fresh RPMI 1640 growth medium (1 ml) with cyclosporin A once a week without removal of spent medium.

   *Maintain cultures as outlined in Inoculation of Flasks for Mammalian Cell Suspension Cultures.*

REFERENCES