SOP: **Namalwa cell culture and infection by Sendai Virus**
Date modified: **03/25/2013**
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Ordering Information

Human B cell line Namalwa can be ordered from ATCC. This is a suspension cell line.

Name: Namalwa—Human B Lymphocyte Cells
ATCC #: CRL-1432

Materials List

1. RPMI-1640 medium with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate, 4.5g/L glucose, 10mM HEPES, and 1.0mM sodium pyruvate, (ATCC, Cat# 30-2001)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. Penicillin-Streptomycin Solution (200X) (Corning/Cellgro, Cat# 30-001-CI)
4. Sendai Virus Cantell strain (Charles River Laboratories, Cat# 10100772, viral titer > 2000 HAU/mL)
5. T75 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated pipets (1, 5, 10, 25, 50mL)
8. Phosphate Buffered Saline (1X PBS) (Corning/Cellgro, Cat# 21-040-CM)
9. Freezing Medium (Growth medium containing 10% DMSO)
10. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
11. Cryovials (Nunc, Cat# 368632)
12. Cryo 1°C Freezing Container (Nalgene Cat# 5100-0001)
13. Eppendorf Centrifuge 5810R
14. Revco UltimaII -80°C Freezer
15. Thermolyne Locator 4 Liquid Nitrogen Freezer
16. Hemocytometer
17. Micropipet w/ P20 tips
18. Microscope

Growth Medium for Namalwa

RPMI 1640 medium with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate, 4.5g/L glucose, 10mM HEPES, and 1.0mM sodium pyruvate (92.5%)
Characterized FBS (7.5%)
Pen-Strep (1X)

Cell Culture Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2) When ready to start cell culture, quickly thaw the vial in a 37°C water bath.

3) As soon as ice crystals disappear, swab outside surface of the vial with 70% ethanol, then dispense contents of the vial into a 75cm^2 tissue culture flask and dilute with 40mL complete culture medium.
4) To incubate the culture, place the flask in a 37°C, 5% CO_2 humidified incubator.

B. Sub-culture

1) Propagate cells until density reaches 2 million cells/mL.
2) Transfer 10mL of Namalwa culture into a new 75cm^2 tissue culture flask, and dilute with 30 mL of complete culture medium.
3) Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

1) Namalwa cells can be passaged every 3-4 days. Use 40mL of growth medium per 75cm^2 tissue culture flask.
2) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured and a small portion should be set aside as a seed stock. The cell pellet (500 x g for 5 minutes) for the seed stock should be resuspended in freezing medium.
3) Cells in freezing medium are dispensed into cryovials (2 million cells per 1 mL aliquot) and frozen at -80°C in a Nalgene Cryo 1°C freezing container overnight.
4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Harvest

1) Passage cells until the desired number of cells is reached.
2) According to protocol described above under "Maintenance and Generation of Seed Stocks," collect the cell pellet as for a seed stock (500 x g for 5 minutes).
3) Wash cell pellet by resuspension in PBS.
4) Examine viability using Trypan blue staining (SOP TP-7).
5) Collect cells by 500 x g 5 minutes centrifugation.

E. Sendai Virus infection

1) Start Namalwa culture as described above.
2) Thaw the stock Sendai Virus from the frozen vial (aliquoted upon receiving) in a 37°Cwater bath.
3) Start infection by adding the virus directly to the Namalwa culture flask to a final concentration of 200 HAU/mL. It will be ideal to infect cells at a density of 1 million cells/mL.
4) Continue to culture cells in a 37°C, 5% CO_2 humidified incubator for desired time.
5) Harvest cells by spinning at 500 x g for 5 minutes in Corning conical centrifuge tubes (15mL or 50mL).
6) Wash cell pellets by resuspension in PBS, and collect cells by 500 x g 5 minutes centrifugation.
7) Perform Percoll gradient purification according to **SOP: Cell purification using Percoll step gradients (04/01/2013).**
8) Freeze purified Namalwa cells according to **SOP: Cryopreservation of hematopoietic cells from human leukapheresis product (02/07/2011).**