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DATA SHEET

Reagent:	SIV B670 Virus
Catalog Number:	633
Lot Number:	Lot 2
Release Category:	C
Provided:	1 mL of cell-free virus
Original Source:	Isolated from a rhesus macaque monkey (#B670) with AIDS-like disease. Propagated in primary human PHA lymphoblasts and harvested from supernatant.
Host Strain:	Rhesus peripheral blood monocytes and lymphocytes. Also infects human peripheral blood monocytes and lymphocytes, CEM×174, CEM-SS, and H9.
Sterility:	Negative for mycoplasma, bacteria, and fungi
Description:	A lab-adapted SIV isolate
Special Characteristics:	This virus is highly pathogenic for primates. It must be handled with utmost caution. Infected primates show high incidence of retroviral encephalitis. Alternate Name: STLV-III/Delta
Recommended Storage:	Keep the reagent in liquid nitrogen.
Contributor:	Dr. Michael Murphey-Corb

References:

Baskin, G. B., Murphey-Corb, M., Watson, E. A., & Martin, L. N. (1988). Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol, 25(6), 456-467. doi:10.1177/030098588802500609 PUBMED

Murphey-Corb, M., Martin, L. N., Rangan, S. R., Baskin, G. B., Gormus, B. J., Wolf, R. H., . . . Montelaro, R. C. (1986). Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature, 321(6068), 435-437. doi:10.1038/321435a0 PUBMED

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: SIV B670 Virus from Dr. Michael Murphey-Corb." Also include the references cited above in any publications.

Preparation of Cryopreserved Pathogenic SIV/Delta$_{B670}$ Virus Stock.

Dr. Michael Murphy-Corb, Delta Regional Primate Research Center, Tulane University, Covington, LA.

The following procedures may be used for routine propagation of SIV/Delta$_{B670}$ in human or rhesus peripheral blood mononuclear cells (PBMC). The virus can also be passed in a number of established cell lines such as H9, CEM-SS, and CEM×174, but PBMC are preferred to maintain pathogenicity. Note that SIV should be handled with utmost caution because it is highly pathogenic in monkeys..

Reagents.

PHA-Stimulation Medium RPMI 1640 supplemented with 15% fetal bovine serum, 10 µg/ml PHA, 2 µg/ml Polybrene.

Culture Medium 75% RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 50 µg/ml streptomycin; 10% human IL-2 (Pharmacia ENI, catalog #6010 and #6011); 15% fetal bovine serum.

Wash Medium RPMI 1640 with 15% fetal bovine serum.

SIV/Delta$_{B670}$ Virus Repository catalog #633.

A. Preparation of PHA-Stimulated Human or Rhesus Peripheral Blood Mononuclear Cells.

1. Collect 30 ml of heparinized whole blood (minimum). Isolate PBMC using Ficoll-Hypaque. The yield from this isolation should be approximately 3 - 4 x 10^7 PBMC..

2. Wash the cells three times using 5 ml of wash medium per wash. Pellet the cells at 500 g for 10 minutes at room temperature. Save 100 µl of the suspended cells at the time of the third wash for viability counts (step 3)..

3. Determine the viable cell number by trypan blue exclusion and resuspend the total pool in PHA-stimulation medium at a final concentration of 1 x 10^6 cells/ml..

4. Transfer resuspended cells to a 75cm^2 flask and place in a vertical position at 37°C. To increase the rate of growth, place flask horizontally in incubator..

5. After 3 days, transfer cells to centrifuge tubes. Centrifuge the cells at 500 g for 10 minutes at room temperature..

6. Resuspend the cell pellet in 10 ml of wash medium and wash three times as described in step 2. Determine viable cell number..

Infection of PHA-Stimulated PBMC with SIV/Delta$_{B670}$.

1. Transfer 1 x 10^7 PBMC to a centrifuge tube. Centrifuge cells at 500 g for 10 minutes at room temperature and remove the wash medium..

2. Add 1 ml of cell-free SIV/Delta$_{B670}$ virus (titer 75,000-200,000 cpm; see **Reverse Transcriptase Assay**, below). to the cell pellet. Incubate 1 hour at 37°C..

3. Wash cells twice in 5 ml of wash medium and centrifuge at 500 g for 10 minutes at room temperature. After the second wash, resuspend the cells in 10 ml of culture medium and transfer cells to a 25cm^2 flask, and incubate at 37°C..

4. Every 3 - 4 days, remove 5 ml of culture medium and replace it with 5 ml of fresh culture medium. Maintain the cells at 5 x 10^5 - 1 x 10^6 cells/ml by adding fresh PBMC..

5. Assay for reverse transcriptase (RT) activity in 3 - 4 ml of culture fluid on post-infection days 7, 10, 14, 17, and 21 (see below)..

6. When the culture has an RT value of at least 50,000 cpm (approximately 7 - 14 days post-infection), expand the culture by supplementing the uninfected cells with fresh uninfected PHA-stimulated PBMC at a ration of 1:1 (uninfected cells:infected cells). Continue the incubation at 37°C..

7. Determine the viable cell number and assay RT activity every 3 - 4 days. Maintain the cell density at 5 x 10^5 - 1 x 10^6 cells/ml by adding fresh PBMC and culture medium..

8. Harvest the virus when the RT activity is increasing and not yet at a peak value (usually at 10 - 14 days post-infection) as follows:.

a. Centrifuge the cells at 500 g for 10 minutes at room temperature..

b. Collect supernatant and pass it through a 0.22 µm filter..

c. Aliquot supernatant into 1 ml volumes and store in liquid nitrogen..

Reverse Transcriptase Assay.

Reagents

PEG Solution 0.4 M NaCl, 30% polyethylene glycol 8000 (PEG)

Dissociation Buffer 0.5% Triton X-100; 0.8 M NaCl; 0.5 mM phenylmethylsulfonyl fluoride; 20% glycerol; 50 mM Tris-HCl (pH 7.8)

poly (ra)p(dT)$_{12-18}$ (Pharmacia LKB Biotechnologies, Inc., catalog #27-7878-02)

[^{3}H] TTP (50-80 Ci/mmol in 10 mM Tricine, Dupont NEN, catalog #NET-221A)

Stop Solution 2.5 mg/ml tRNA; 10 mM Tris (pH 7.8); 100 mM NaCl,1.0 mM EDTA

TCA Precipitation Solution 10% trichloroacetic acid (TCA); 0.2% sodium phosphate

Wash Solution 5% TCA; 0.02% sodium phosphate

Ethanol 70%

A. Virus Precipitation

1. Clarify 3 - 4 ml of cell culture supernatant by centrifugation at 8000 g for 10 minutes at 4°C.

2. Transfer 3 ml of the supernatant to a clean tube and add 1.5 ml PEG Solution. Mix and incubate on ice in a cold room overnight.

3. Centrifuge at 800 g for 45 minutes at 4°C. Pour off supernatant; carefully remove last drops with a pipet.

4. Freeze remaining PEG pellet at -70°C until ready to assay.

B. Reverse Transcriptase Assay

1. Disrupt the precipitated virus as follows:

a. Transfer PEG pellet from -70°C freezer immediately to an ice bucket. Add 100 µl cold dissociation buffer.

b. Resuspend pellet by repeated pipeting. Avoid bubbles.

c. Incubate 10 minutes on ice for complete disruption.

2. Immediately before use, prepare the following reaction mixture in a 500 µl microfuge tube:

Stock Solution Final Volume Concentration 300 mM Tris, pH 7.8 31.2 µl 52 mM 42 mM MgCl2 43.0 µl 10 mM 42 mM dithiothreitol 21.5 µl 5 mM 0.9 µg/10 µl poly (rA)p(dT)12-18 10.0 µl 5 µg/ml 20 mM dATP 1.26 µl 83 µg/ml 1 mCi/0.4 ml [3H] TTP 3.74 µl 52 µCi/ml H2O 69.3 µl Final Volume: 180.0 µl

3. Transfer 20 µl of disrupted virus to the microfuge tube containing the reaction mixture and mix gently. Incubate at 37°C for 2 hours. Include a positive control (virus with known RT activity) and a negative control (supernatant and uninfected PBMC).

4. Add 10 µl Stop Solution to each sample.

C. TCA Precipitation

1. Transfer the entire sample to a tube containing 3 ml of cold wash solution. Incubate on ice 30 - 40 minutes to precipitate DNA.

2. Pre-wash glass fiber filter in a suction apparatus with cold wash solution.

3. Pour contents of tube onto filter and suction through apparatus. Rinse tube twice with wash solution and suction through apparatus.

4. Wash filter 4 times with cold wash solution and once with cold 70% ethanol. Remove filter from suction apparatus and allow to air dry.

5. Put dried filters into small (17 mm diameter) scintillation vials, add ml of scintillation cocktail to each vial and count.

Last Updated: June 30, 2017