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DATA SHEET

Reagent:	U373-MAGI Cells
Catalog Number:	3595
Lot Number:	020008
Release Category:	B
Provided:	1 x 10^6 cells/vial.
Cell Type:	U373MG cells
Propagation Medium:	DMEM 90%; fetal bovine serum, 10%; 0.2 mg/ml G418; 0.1 mg/ml hygromycin B.
Freeze Medium:	Propagation medium, 90%; DMSO, 10%.
Growth Characteristics:	Adherent cell line; split 1:5 once or twice per week. The cells do not grow well when split too thinly.
Sterility:	Negative for mycoplasma, bacteria and fungi.
Description:	U373MG cells expressing CD4
Special Characteristics:	These cells can be used as a control in a focal immunoassay for titration of HIV-1, HIV-2, and SIV isolates. CD4 is linked to neo^r and the HIV-1-LTR-ß-Gal sequence linked to $hygro^r$. **Reference Protocol:** Protocol: Determining coreceptor usage of virus isolates
Recommended Storage:	Liquid nitrogen.

Contributor: Dr. Michael Emerman and Dr. Adam Geballe.

References:

Vodicka MA, Goh WC, Wu LI, Rogel ME, Bartz SR, Schweickart VL, Raport CJ, Emerman M. Indicator cell lines for detection of primary strains of human and simian immunodeficiency viruses. *Virology* **233**:193?198, 1997.

Harrington RD, Geballe AP. Cofactor requirement for human immunodeficiency virus type 1 entry into a CD4-expressing human cell line. *J Virol* **67**:5939?5947, 1993.

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: U373-MAGI from Dr. Michael Emerman and Dr. Adam Geballe." Also include the references cited above in any publications.

Research Chart: This protocol describes a method for titering and determining coreceptor usage of viral isolates. The method uses three different cell types that either express no added coreceptor (U373-MAGI) or express CXCR4 alone (U373-MAGI-CXCR4cem) or CCR5 alone (U373-MAGI-CCR5e) in the presence of CD4 and the indicator gene. Infected cells and syncytia are easily visualized under light microscopy by their blue nuclear staining.

Reagents

Cells U373-MAGI and derivatives U373-MAGI-CXCR4 and U373-MAGI-CCR5e (Catalog #3595, 3596, and 3597, respectively).

Test Virus HIV-1, HIV-2, or SIVmac strains that use either CXCR4 or CCR5 as coreceptors.

DEAE Dextran (Pharmacia, Catalog #17-0350-01), prepared at 200 ?g/ml in DMEM.

Culture Medium DMEM 90%; fetal bovine serum, 10%.

Fixing Solution 1% formaldehyde, 0.2% glutaraldehyde (in PBS). This solution can be made up in advance and stored at 4?C in the dark for approximately 1 month.

Staining Solution To prepare 1.0 ml of Staining Solution, combine 950 ?l PBS, 20 ?l 0.2 M potassium ferrocyanide, 20 ?l 0.2 M potassium ferricyanide, 1.0 ?l 2.0 M Mg_2Cl, and 10 ?l X-gal Stock.

X-Gal Stock Prepare at 40 mg/ml in DMSO. X-gal stock should be stored in the dark at -20?C. It will turn yellow over time, but this does not affect the assay. Discard the stock if it becomes greenish-brown.

Procedure

1. Plate U373-MAGI cells at 0.6 x 10^5 cells per well (24 well plate) or at 1.2 x 10^5 cells per well (12 well plate). The cells should be 30% confluent one day after plating.

2. One day after plating out the cells, prepare dilutions of test virus in culture medium. Remove the culture medium from the plated cells and add 150 ?l of virus to each 6 mm well (300 ?l if a 12 well plate is used). Virus samples should be tested in duplicate. Add DEAE Dextran to each well at a final concentration of 20 ?g/ml.

3. Allow the virus to adsorb for at least 2 hours in a 37?C, 5% CO_2 incubator. Rock the plates every 45 minutes to prevent the cells from drying.

4. After the adsorption period, add 1-2 ml of fresh culture medium to each well. It is not necessary to remove the viral inoculum. Incubate the cells 40-48 hours in a 37?C 5% CO_2 incubator. The cells should be just subconfluent.

5. Remove the culture medium and add 1-2 ml of fixing solution to each well. Incubate for exactly 5 minutes at room temperature. ?-galactosidase activity decreases dramatically if the fixing solution is left on for more than 10 minutes.

6. Remove the fixing solution and wash the cells twice with PBS. Add enough staining

solution to each well to just cover the cells. Incubate the cells at 37?C for two hours. Do not extend the incubation period or background staining will occur. NOTE THAT THE INCUBATION TIME FOR THE U373-MAGI CELLS IS LONGER THAN THAT FOR THE HELA-MAGI CELLS.

7. Wash the plates twice with PBS. Positive syncytia will stain blue. Most staining will be primarily in the nuclear region because the ?-gal gene has been modified with the SV40 nuclear localizing sequence. Count the number of blue-stained cells. Titration values are expressed as the number of stained cells multiplied by the viral dilution.

8. Plates can be stored in PBS with sodium azide if a permanent record is desired. The color will not fade if the plates are kept from strong sunlight.

Alternate Method for Titering Infected Cells:

Dilutions of infected cells, rather than cell-free virus, can be plated onto the U373-MAGI cells if desired. Prepare serial dilutions of infected test cells in culture medium starting with 1 x 10^5 cells. If infected cells are used, do not add DEAE Dextran to the cultures. It is not necessary to wash off unattached cells from the monolayer.

Last Updated July 03, 2018