**Note: This SOP is taken from the validated SOP from the Duke University Central Reference Laboratory, by David Montefiori et al, with minor modifications.*

Henry Jackson Foundation, USA

Neutnet code: 5B
Contact person: Lindsay Wieczorek, lwieczorek@hivresearch.org

I. Purpose

To infect TZM-bl cells with pseudovirus and measure luciferase expression.

II. Scope

This SOP applies to all qualified employees of the Henry M. Jackson Foundation/USMHRP Humoral Immunology Laboratory (HIL) that will utilize TZM-bl cells.

II. Materials

A. Biological safety cabinet, class II
B. Incubator, 37° C, 5% CO_2
C. Centrifuge equipped with microplate carriers
D. Compound microscope
E. Inverted microscope
F. Hemocytomer
G. Luminometer (Perkin Elmer)
H. PipetteAid & Stripette tips (5ml, 10ml, and 25ml)
I. Single and multichannel pipetters & pipette tips
J. T-75 culture flask
K. Black-bottom, flat-bottom 96-well microplate (Culture Plate 96-F) (Perkin Elmer)
L. Clear-bottom, flat-bottom 96-well microplate (View plates) (Perkin Elmer)
M. 50 ml conical tubes
N. Dulbecco's Phosphate Buffer Saline, (PBS)
O. Complete DMEM containing 15% heat-inactivated FBS, L-glutamine, and penicillin-streptomycin
P. Trypsin (Quality Biological)
Q. 37°C Water Bath (180 Series Precision Scientific, Winchester, VA)
R. DEAE-Dextran
S. Cultured TZM-bl cells.
T. Luciferase Britelite Reconstitution Buffer (PerkinElmer)
U. Luciferase Britelite Substrate (PerkinElmer)
V. Pseudovirus stock
W. Test neutralizing reagents

III. Definitions and Abbreviations

A. TZM-bl = a luciferase expressing reporter cell that possesses CD4 and both CXCR4 and CCR5 coreceptors
B. DMEM = Dulbecco/Vogt Modified Eagle's Minimal Essential Medium
C. cDMEM = complete DMEM media
D. FBS = Fetal Bovine Serum
E. RLU= Relative Light Unit

IV. Procedure

All incubations are performed in a humidified 37°C, 5% CO_2 incubator unless otherwise specified.

TZM-bl Neutralization Assay

1. Dilute test neutralizing reagents to desired concentrations.
2. Add 25µL of test plasma/sera/antibodies to the microplates. The first microplate for each assay should be a black, clear-bottom view plate. Additional microplates can be the black-bottom culture plate.
3. Dilute test pseudovirus(es) to desired concentrations.
4. Add 25µL of pseudovirus to the microplates.
5. Incubate all microplates at 37ºC, 5% CO_2 for thirty minutes.
6. While the microplates are incubating, count and resuspend the TZM-bl cells in cDMEM.
    a. Trypsinize a flask of TZM-bl cells 20 minutes prior to use. Remove all the media from a flask of grown TZM-bl cells with a stripette. Add 5ml of PBS to the flask and lightly swirl for up to one minute. Remove all PBS with a stripette. Add 3mls of Trypsin to the flask and incubate for five minutes at 37ºC, 5% CO_2. Transfer this 3mls of cells to a 50ml conical tube containing 27ml cDMEM. Centrifuge for ten minutes at 1200rpm. Resuspend these cells at $2x10^5$ cells/mL in cDMEM containing a concentration of 60ug/ml DEAE-dextran, final concentration 30ug/ml.
7. When the thirty minute incubation is complete, add 50µL/well of the resuspended TZM-bl cells to the microplates. Return the microplates to incubate at 37ºC, 5% CO_2 for 48 hours.

Analysis of TZM-bl neutralization

1. After a 48 hour incubation period prepare the Britelite substrate for plate analysis.
    a. Reconstitute the lyophilized luciferase Britelite substrate by adding luciferase Britelite reconstitution buffer, 10mls buffer into a 10ml substrate bottle. Mix and keep at room temperature.
2. Before adding substrate to the microplates, observe the clear-bottom view plate under an inverted microscope for confluence.

3. Add 100µl Britelite substrate to all wells of the microplate. Wait one minute before removing the microplates from the hood.
4. Read plates using a Victor luminometer, use the Luminescence plate protocol.
5. Average the RLU value for each dilution of neutralizing reagent and compute the IC50, 80 and 90 values. Record along with percent neutralization.

V. Revision History

05December08- Anita Gillis