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ABSTRACT

Neutralizing antibody (Nab) assays have many applications in the study of human immunodeficiency virus (HIV). These include studying the immune response in infected individuals, monitoring viral diversity and judging vaccine immunogen candidates in both clinical and preclinical trials. To be of any use in these applications, an assay must achieve equivalent results when performed in many different laboratories. The author describes a luciferase reporter gene assay that meets these requirements.

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Introduction

In this chapter of HIV Protocols, the author describes the main endpoint neutralization assay used by NIH-sponsored HIV vaccine trials. The assay is a luciferase (Luc) reporter gene assay that measures HIV neutralization as a function of Tat-regulated Luc reporter gene expression reduction after a single round of HIV infection in TZM-b1 cells(1) (2) . TZM-b1 cells (also called JC53BL-13 cells) are a CXCR4-positive HeLa cell line that expresses the cellular receptor, CD4, and the coreceptor molecule, CCR5(3) . The cell line also contains integrated reporter genes for luciferase and *E. coli* beta-galactosidase, both under the control of an HIV long-terminal repeat sequence (*tat* gene)(4) . The cells are susceptible to infection by a variety of HIV, SIV and SHIV strains, including primary HIV isolates and molecularly cloned Env-pseudotype viruses. The assay is a neutralization assay for single-cycle infection with either uncloned viruses grown in human lymphocytes or molecularly cloned Env-pseudotyped viruses produced by transfection in 293T/17 cells.

> *"The Luciferase activity, quantified as relative luminescence units (RLU), is directly proportional to the number of infectious viral particles present in the initial sample."*

Pseudovirus Production

A detailed description of Env-pseudotype virus production is given in the chapter. Briefly, assay stocks of Env-pseudotyped viruses are produced by cotransfecting 293T/17 cells with an Env expression plasmid and a second plasmid that expresses the entire HIV-1 genome except for Env. Only the *env*-minus plasmid is transcribed into viral genomic RNA that is packaged by the pseudovirions for delivery of the *tat* gene to TZM-b1 cells. This cotransfection approach generates pseudovirus particles that are infectious but can not produce infectious progeny, limiting infection to a single cycle.

Luciferase Assay

Soon after infection with uncloned viruses or cloned Env-pseudotyped viruses, reporter gene expression is induced by the viral Tat protein. The luciferase activity, quantified as relative luminescence units (RLU), is directly proportional to the number of infectious viral particles present in the initial sample. The assay is sensitive over a wide range of values and can be performed in 96-well plates for higher-throughput assays.

The chapter diagrams a plate layout for measuring the titer of Nabs in five samples in duplicate at eight dilutions on a single 96-well plate. A second layout is provided for screening preimmune and postimmune samples from 20 study subjects on a single plate. Typical positive samples show neutralization curves that are linear in the range of 20–85% reductions in RLU. For best results, the Nab titer is defined as the 50% inhibitory dose (ID50), which is in the middle of the linear portion of the neutralization curve. Finally, automated reduction is possible using the plate layouts provided in the chapter and publicly available Excel macros.

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