VIRAL TITER COUNTS

The best methods of measuring infectious lentiviral titer
Summary

Lentiviral vectors are routinely used for gene transduction in a wide variety of experimental protocols. The success or failure of these experiments often hinges on the efficiency of transduction. Partial gene knockouts, intermediate siRNA knock downs, and mixtures of transduced and non-transduced cells expressing transgenes leads to unacceptably ambiguous results in basic research and unusable commercial products. Across the globe, time and effort is spent each day parsing why a particular gene transduction did not achieve an acceptable efficiency. One of the culprits is often a lower than anticipated infectious titer, and much effort is spent attempting to improve this parameter.

It has long been known that in lentiviral suspensions the ratio of active, infectious virus to total viral particles is variable, and can be unfavorably low (1-5). Because of this, simple particle counts are generally not a guide to infectious titers. Consequently, several methods have gained acceptance as useful measures of infectious viral titer.

In this report, we look at the pros and cons of the most commonly practiced methods for determining infectious virus titer, along with a newer approach that delivers both speed and accuracy.
THE NEED TO MEASURE VIRAL TITER

Mammalian viruses are used as reagents for the study of virus and host biology, in drug discovery programs, as vehicles for gene transfer and for regulating gene expression.

In these and other applications, knowledge of the infectious viral titer – along with the ratio of infectious to non-infectious particles – can make the difference between success and failure; between clarity and confusion.

Do you know what’s going into your experiment?

You are developing a passive immunotherapeutic and testing it against stocks of your target virus. Here, inaccurate knowledge of infectious titer from aliquot to aliquot can easily lead to meaningless observed variations in antibody effectiveness, significant wastes of time and erroneous conclusions.

You are using a viral vector to help identify and clone an important new gene. Failing to accurately quantitate the true infectious titer of your cDNA clone stock can result in your using an MOI that’s too small, and missing your target gene; or an MOI that’s too large, and transducing more than one gene per cell. In either case, you don’t get your gene and waste enormous amounts of time.

You are using a gene cloned in a virus to knock down gene expression in the host cell. The level of knock down you observe will depend heavily on controlling the percent infection you achieve. If you do not know the true infectious titer of your viral stock, you may never be able to obtain an accurate understanding of the true effects of gene suppression in your system.
MEASUREMENT BY PROXY – COMMON NON-FUNCTIONAL METHODS

The quickest methods used to estimate infectious viral titer take advantage of viral proxy molecules in order to avoid the lengthy times associated with cell growth and viral replication. These methods focus on quantitating the presence of key viral components – RNA and capsid protein.

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<tr>
<th>Measurement by Proxy</th>
<th>common non-functional methods</th>
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<td><strong>qPCR of Viral RNA</strong></td>
<td><strong>P24 Quantitation by ELISA</strong></td>
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<th><strong>PRO</strong></th>
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<td>This method is fast (get a time estimate), sensitive (get a sensitivity estimate) and accurate. While it requires access to qPCR instrumentation, most labs have that capability in place or can get to it easily.</td>
<td>Does not distinguish RNA that comes from infectious virions from free viral RNA in the suspension. Because of this, qPCR tends to overestimate the infectious viral titer, even by orders of magnitude.</td>
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<td>A variety of commercially available ELISA kits provide fast, easy and reliable quantitation of p24. Moreover, ELISA readers are inexpensive and common to most cell biology and immunology labs.</td>
<td>Does not distinguish between measurement of p24 that is authentically assembled into a viral capsid and free p24 that has been released into the media and is not associated with an infectious particle. Just as with RNA quantitation, viral protein quantitation tends to overestimate the infectious viral titer significantly.</td>
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Direct measurement of the infectivity of the viral suspension has been the method of choice. For lentiviral vectors the analysis typically relies on reporter molecule activity in the infected cell. The resultant data indicates infectious unit per volume of media.

This procedure is time consuming, but accurate. Host cells are infected with serially diluted virus suspension, cells are grown, harvested and the infected cells are quantitated by flow cytometry or in situ luciferase or galactosidase assays. Measurements made from multiple plates will give a reliable estimate of the infectious titer.

**PRO**

This functional method will give an accurate estimate of the true infectious titer, since it requires successful infection for the measurement.

**CON**

1) If the titer is low, this assay provides no information for troubleshooting. Did the procedure to produce this virus fail because little virus was made (perhaps due to a poor transfection), or was amount of virus reasonable but the ratio of infectious to non-infectious unfavorable? In vivo, a high ratio of non-infectious to infectious viruses can induce interferon dependent defense that causes the experiment to fail.

2) In the case where drugs, vaccines or passive immunotherapeutics are being developed against a viral target, the infectious titer is misleading, because the drug or antibody will bind to the intended viral protein whether or not that protein is part of a virus.
RT ANALYSES THE BEST OF BOTH WORLDS

Endogenous viral reverse transcriptase activity provides a measurement option that combines the benefits of speed and accuracy. The method has the simplicity and speed of a biochemical assay, but still reports only on viable, infectious virus.

The reverse-transcriptase assay achieves its accuracy in estimating infectious titer because it measures enzyme activity that can only be part of a viable, infectious virus. In this sense, it is the equivalent of a plaque forming assay or the flow cytometric assay of infected cells.

The benefits of measuring reverse transcriptase as a surrogate for viable virus include:

- Fast turn-around time
- Simple, reliable protocol
- Accurate estimate of true viral infective titer
- Can be automated
- Time and cost effective
- It empowers the user to optimize and troubleshoot their own viral preparations.
About the Cavidi RT kits

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<th>Kit</th>
<th>Description</th>
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<tr>
<td>Lenti RT Activity Kit</td>
<td>High sensitive RT assay optimized for HIV-1, HIV-2, SIV, FIV and Visnavirus retroviruses</td>
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<tr>
<td>C-Type RT Activity Kit</td>
<td>High sensitive RT assay optimized for C-type retroviruses</td>
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<tr>
<td>HS-Lenti RT Activity Kit</td>
<td>Ultrasensitive RT assay optimized for HIV, SIV and FIV retroviruses</td>
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<td>HS-MN RT Activity Kit</td>
<td>Ultrasensitive RT assay optimized for Mn2+- dependent retroviruses</td>
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<tr>
<td>HS-MG RT Activity Kit</td>
<td>Ultrasensitive RT assay optimized for Mg2+-dependent retroviruses</td>
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<tr>
<td>XMRV Test Kit</td>
<td>Highly sensitive RT assay for the study of XMRV</td>
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<tr>
<td>DNA Polymerase Assay Kit</td>
<td>Outstanding sensitivity on an easy to run platform</td>
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For detailed information about components, applications and pricing of these kits, please visit http://www.cavidi.se/enzyme-assays/rt-kits/

References


About Us

Cavidi is Sweden’s leading supplier of viral load for HIV monitoring. Cavidi’s products enable efficient management of anti-HIV drugs (ARV therapy) so that people living with HIV can live a normal lifespan. Cavidi’s diagnostics reduce the cost of treatment and the spread of HIV. Cavidi also provides tools and kits for virus research to many of the world’s leading labs.

If you would like more information about Cavidi or our products, please contact us directly.

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