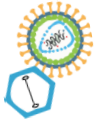
How To TITER VIRUS

Methods to Measure Lentivirus and Adeno-associated virus (AAV) in Your Sample

Mirus



TITER: *from the French word 'tiltre,' which historically referred to the amount of gold in coin and other gold alloys.*

Just like measuring the quality of gold products, determining **virus titer** is essential for understanding the value or infectious potential of your samples. Luckily, several established methods exist for reliably quantifying either viral proteins/genomes (*physical* titer, P) or infectivity (*functional* titer, F) of virus-containing samples (Table 1).

What is TRANSDUCTION?

Transduction is the virus-mediated delivery of nucleic acids into eukaryotic cells. Contrast this with the term "transfection," which is more commonly used to describe nucleic acid delivery via *non*-viral methods.

Transient transfection of HEK 293 cells (i.e. a packaging cell line) with plasmids is often used to produce the virions that will then be used for transduction of target cells.

Table 1. Lentivirus and AAV Titering Methods

Titer Type: Physical (P) or Functional (F)

Method	Measurement	Units	Notes	
Flow cytometry	number of transduced cells	transducing units (TU)/ml	Infectivity is measured by the number of transduced cells expressing viral genes.	F
	number of viral particles	virus particles/ml	Viral particles immobilized on beads or antibodies are counted.	P
qPCR/ddPCR	molecules of lentiviral RNA	genome copies/ml	The quantity of viral genomes within harvested virus samples is measured.	P
	molecules of AAV DNA			
	copies of integrated lentiviral DNA	copies/cell	Infectivity is determined by measuring the quantity of viral genomes in transduced cells.	F
	copies of replicated AAV DNA			
ELISA	viral proteins (*e.g.* capsid epitope)	varies, typically pg/ml	Readout relies on antibody binding directly or indirectly to viral protein.	P
Surface Plasmon Resonance	viral protein binding	virus particles/ml	Changes in the refractive index of a surface upon binding of virions is measured.	P
Tunable Resistive Pulse Sensing	number of viral particles	virus particles/ml	Size and concentration of single particles is measured as they pass through a nanopore.	P
Electron microscopy	number of viral particles	virus particles/ml	Viral particles are visualized and counted.	P

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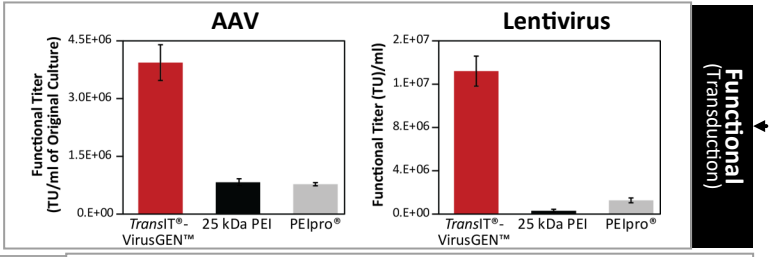
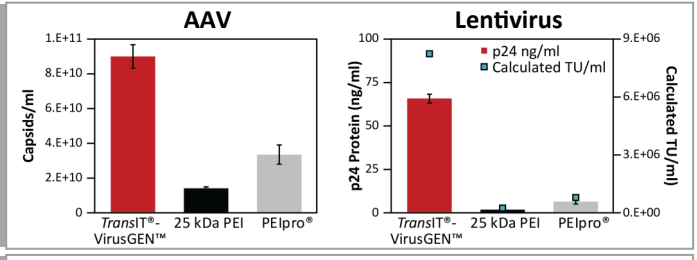
Does HOW you titer matter?

Yes! After transfecting suspension HEK 293 cells with *Trans*IT®-VirusGEN™, 25 kDa PEI or PEIpro®, the same preps were titered with three different methods. While relative titers (i.e. *Trans*IT®-VirusGEN™ > PEIpro® > 25 kDa PEI) are consistent across methods, only functional titers directly correlate to the prep's ability to transduce cells. Conversely, using transfection efficiency is the least reliable titering method as it does not necessarily reflect whether the prep contains infectious particles.

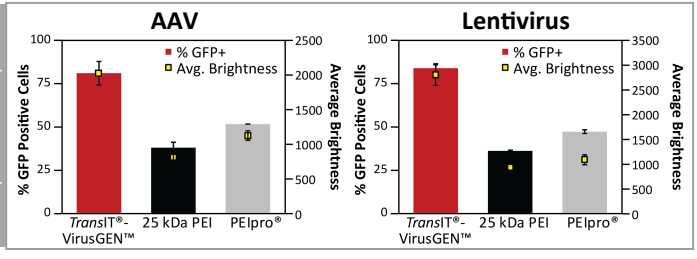
Functional (Transduction)



Viral Protein (ELISA)



Expression of Transfer Vector (Transfection)



For more information, please visit: https://www.mirusbio.com/VirusGEN.

TITERING BY TRANSDUCTION

an illustrative example

Transfection

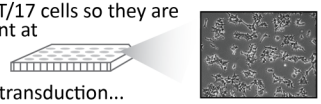
Lentivirus was collected after transient transfection. In this example, the transfer vector encodes GFP.

What's the titer?

To obtain a functional titer, flow cytometry of transduced cells can be performed.

Transduction

1. Plate HEK 293T/17 cells so they are ≥ 40% confluent at transduction.



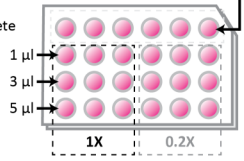
2. On the day of transduction...

Trypsinize and count at least 2 wells, e.g. mean = 2 x 10^5 cells.

In remaining wells, carefully replace half of the media with complete growth media containing *Transduce*IT™.

3. Dilute lentivirus stock in complete growth media, e.g. 5-fold or 0.2X.

Add the indicated volume of virus to the wells of each row.



For an accurate titer, < 20% of cells should be GFP+ at 72 hr post-transduction. This minimizes the chance a cell will be transduced by more than one virus. Thus, testing several dilutions is recommended.

Analysis

4. Assay the cells for GFP expression by flow cytometry 72 hr post-transduction.

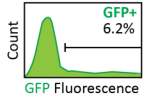
5. Example Calculation:

Using data, averaged, from wells that received 5 µl of 0.2X virus:

'% GFP+ cells x 'Total # of cells (from Step 2)'

= 6.2% x 2 x 10^5 cells

= 12,400 GFP+ cells



Considering that the virus was diluted 5-fold (Step 3), the 5 µl (i.e. 0.005 ml) of virus stock has...

12,400 GFP+ cells x 5 ÷ 0.005 ml = **1 x 10^7 Transducing Units/ml**

Why do HIGH TITERS matter?

Some cell types require application of virus at high **m**ultiplicity **o**f **i**nfection (**MOI**) in order to be transduced.

MOI = transducing units (TU) ÷ number of cells

For example, primary T cells might require virus to be added at an MOI of 5 to result in target gene expression, while cells that are more susceptible to infection might only require an MOI of 1. An MOI of 5 for 1×10^6 cells requires 5×10^6 TU of virus; in other words, 0.5 ml of a virus with a titer of 1×10^7 TU/ml would be required.