Physical particles (PP) determination

Vectalys is an R&D company with a state-of-the-art technology platform for customized viral vector production.

The company has developed a unique process enabling the production of high titer high purity lentiviral vectors for optimal knock-down or over expression in relevant models: primary and stem cells for target gene validation and specific tissue for animal models.

Method of titration

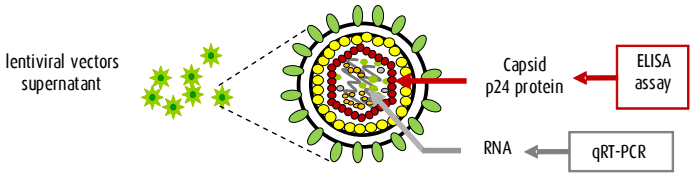
Titers of lentiviral vectors as well as wild type viruses critically depend on the method of titration. The titer can be estimated by the detection of viral physical particles (PP).

In this case, the titer obtained also includes non transduction efficient particles.

The titer is determined by the detection of viral elements directly in the viral supernatant.

> the **p24** viral capsid protein is quantified by **ELISA assay**

> the viral **RNA** is quantified by **qRT-PCR**



Quantification of the p24 capsid protein by ELISA assay

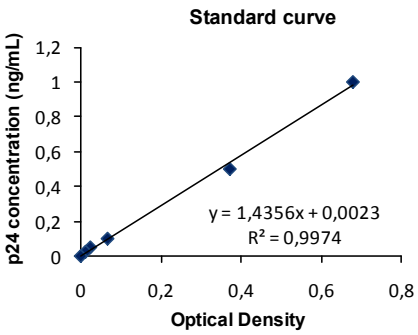
The p24 core antigen is detected directly in the lentiviral supernatant with a HIV-1 p24 ELISA kit (Perkin Elmer).

Viral vectors are lysed with a detergent. The samples are then neutralized and transferred to microplate wells coated with a highly specific mouse monoclonal antibody to HIV-1 p24. The immobilized monoclonal antibody captures both free HIV-1 p24 and that which has been released upon disruption of the vectors in the sample.

The captured antigen is complexed with biotinylated polyclonal antibody to HIV-1 p24, followed by a streptavidin-HRP (horseradish peroxidase) conjugate. The resulting complex is detected by incubation with ortho-phenylenediamine-HCl (OPD) which produces a yellow color that is directly proportional to the amount of p24 captured.

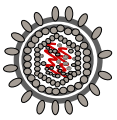
The absorbance of each microplate well is determined using a microplate reader and calibrated against the absorbance of an HIV-1 p24 antigen standard curve.

The number of physical particles is calculated from the p24 concentration with the knowledge that 1pg of p24 corresponds to 1E4 physical particles.



This method allows the detection of all the physical particles, whether functional or not (i.e. immature forms, empty particles) as well as free p24 proteins in the supernatant.

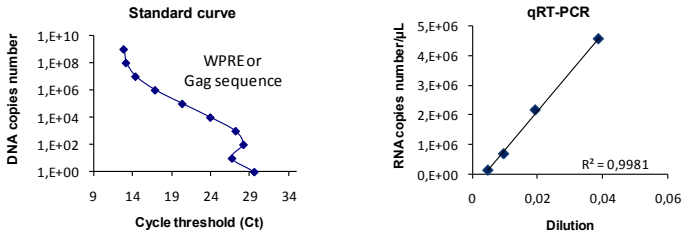
Quantification of the viral RNA by qRT-PCR



Several dilutions of the viral supernatant are performed.

Viral RNA is extracted from the viral particles using a viral RNA extraction kit (QIAGEN).

Reverse transcription is then performed on the RNA fragments obtained and the number of copies of DNA generated is determined by quantitative PCR thanks to a vector specific sequence standard curve.



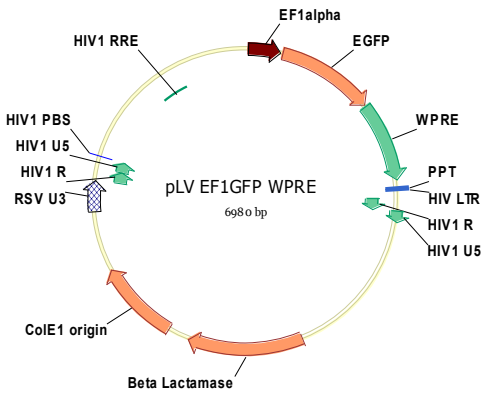
The result obtained critically depends on the conditions of the titration experiment. Indeed, the major variability may be observed during the RNA extraction. The titers also depend on other parameters such as oligonucleotides sequences, PCR conditions… It is therefore important to include standard controls with the samples to standardize the titers from one experiment to another.

This technique allows the detection of all the physical particles containing viral RNA, whether functional or not (i.e. immature forms).

Comparison between titration methods

Titers determined by p24 ELISA assay or viral RNA quantification are therefore an overestimate in terms of the functional titer of the viral vectors. The PP/TU ratio between physical particles and functional ones (transducing units) determines the quality of the viral supernatant. This ratio varies considerably from one production process to another.

Our process allows the production of crude viral supernatants with a PP/TU ratio of around 100-200, demonstrating the high level of quality. Moreover this ratio decreases with increasing level of purification.



A **GFP expressing standard viral vector** is used to correlate the viral titer obtained by p24 ELISA assay or viral RNA quantification (biological titer) with that obtained by FACS (functional titer). It is then possible to use the PP/TU ratio to deduce the functional titer from the viral titer obtained by these techniques for other samples. This is the case if both the expression cassette and the production process are the same as those used by Vectalys (transfection conditions, purification process...).