

Adenoviral titration by quantitative PCR

Rapidly determine adenoviral titers with our Adeno-X qPCR Titration Kit

- Harvest, titrate, and infect in a single day
- Measure titers from crude lysate or purified viral preps

Introduction

The [Adeno-X qPCR Titration Kit](#) provides an extremely fast, simple, and accurate method for titrating adenoviral stocks from all Ad5-based adenoviral vectors. The kit delivers results in just four hours, a vast improvement over standard titration methods (such as the plaque assay), which require up to 10 days to complete. Because qPCR titration is so fast, target cells can be infected with accurately titrated virus on the same day the virus is harvested.

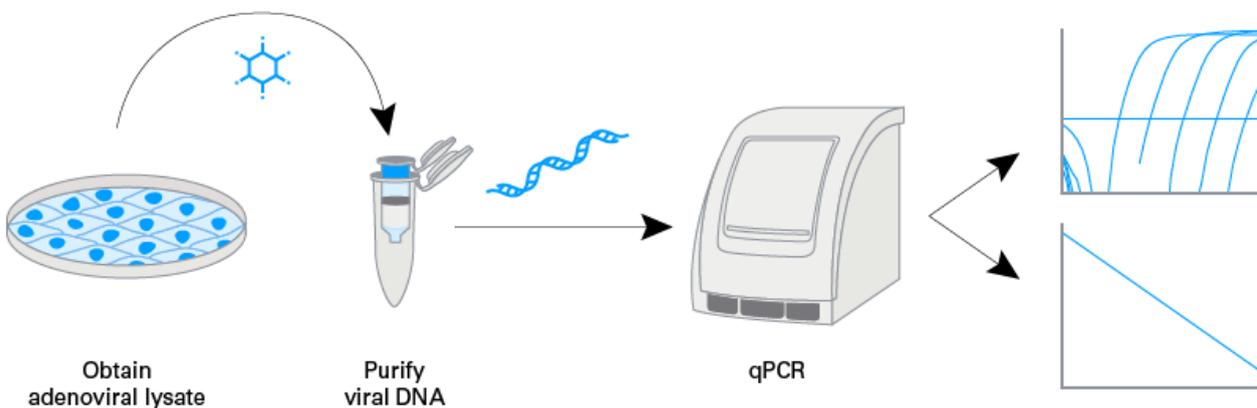


Figure 1. Flowchart of the procedures used for titrating adenoviral DNA with the Adeno-X qPCR Titration Kit.

Results

The qPCR titration method

The protocol combines qPCR with TB Green chemistry, allowing you to determine the viral genome copy number in adenoviral preparations (i.e., crude lysates or purified stocks) from a calibrated DNA standard curve (Figure 2). The procedure is simple: viral DNA and control DNA (provided) are serially diluted and subjected to qPCR. The DNA copy number of each viral sample is then determined by comparing its Ct value to a standard curve generated by plotting the Ct values of the diluted control samples against their respective copy numbers (Figure 2). Titration assays demonstrating the consistency of this approach when used with either crude lysate or purified virus are shown in Figure 3.

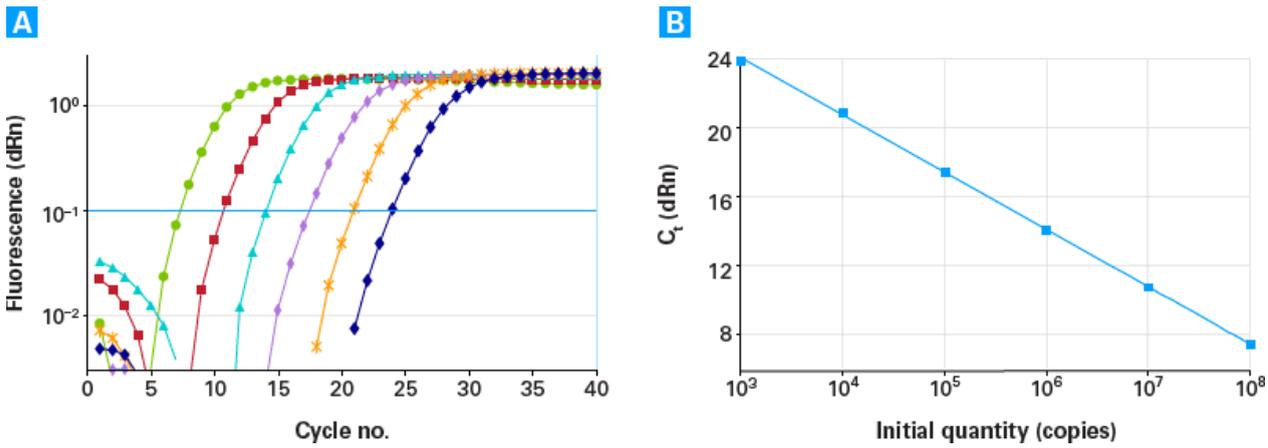


Figure 2. The Adeno-X qPCR titration method exhibits a wide dynamic range. Adeno-X DNA Control Template was serially diluted to 10⁸–10³ copies per sample and analyzed with the Adeno-X qPCR Titration Kit. The amplification plots (**Panel A**) show a dynamic range of at least six orders of magnitude (each dilution is represented by a different colored plot). The standard curve (**Panel B**) demonstrates a strong linear correlation between the Ct and the DNA copy number (log scale), with R² = 1.000 and a PCR efficiency of 96.2%.

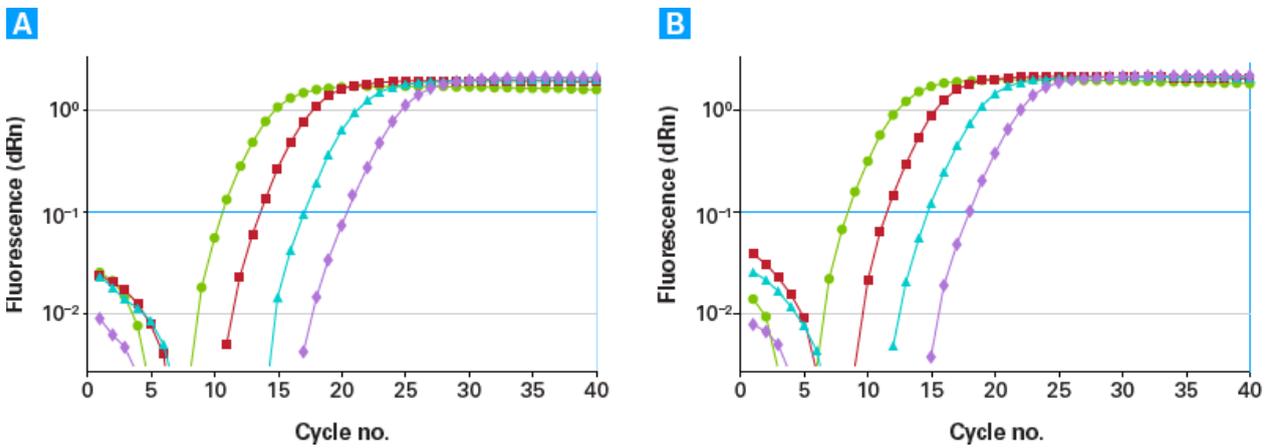


Figure 3. Titrate your adenovirus from crude lysate or purified viral particles. The Adeno-X qPCR Titration kit was used to titrate capsid-free viral DNA obtained from both crude lysate (**Panel A**) and purified viral particles (**Panel B**). In both cases, the capsid-free DNA was obtained with a virus purification kit (provided with the Adeno-X qPCR Titration Kit) and serially diluted (10X) before qPCR (each dilution is represented by a different colored plot). The titration procedure worked equally well regardless of the purity of the viral particles.

Once the genome copy number of your viral stock is determined, it can be correlated with the number of viral infectious units (IFU; determined independently) to establish a copy number/IFU relationship (Table I). Determination of the copy number/IFU relationship for a given prep allows you to normalize the amount of the prep used in each experiment, for consistent interassay results.

Table I: Comparison of Adeno-X qPCR titration to other titration methods

Sample type	Virus	Titration method			Adeno-X qPCR titration ratios	
		qPCR ^a (copies/ml)	Fluor ^b (IFU/ml)	X-gal (IFU/ml)	qPCR/Fluor (copies/IFU)	qPCR/X-Gal (copies/IFU)
Purified	AdAcGFP1	5.62×10^9	3.03×10^9	N/A	2	N/A
Crude	AdAcGFP1					
	Prep A	1.44×10^{10}	1.90×10^9	N/A	8	N/A
	Prep B	1.46×10^{10}	2.26×10^9	N/A	6	N/A
	Prep C	1.38×10^{10}	2.73×10^9	N/A	5	N/A
Purified	AdLacZ	1.01×10^{10}	N/A	1.54×10^9	N/A	7
Crude	AdLacZ					
	Prep A	1.33×10^{10}	N/A	2.67×10^9	N/A	5
	Prep B	1.24×10^{10}	N/A	2.67×10^9	N/A	5
	Prep C	1.21×10^{10}	N/A	3.15×10^9	N/A	4

¹ Adenoviral copy numbers were determined using the Adeno-X qPCR Titration Kit (Cat. # 632252).

² To determine fluorescence-based infectivity titers, adenoviral stocks were serially diluted (tenfold) and applied to HEK 293 cells. After 48 hr, fluorescent cells were scored with a fluorescence microscope.

Conclusions

Our method makes it possible to infect cells at a known multiplicity of infection (MOI), allowing you to produce results that are precise, consistent, and interpretable. The [Adeno-X qPCR Titration Kit](#) includes sufficient material for 200 qPCR reactions and allows for multiple virus preparations to be titrated simultaneously.



Related products

Cat. #	Product	Size	License	Quantity	Details
632252	Adeno-X™ qPCR Titration Kit	Each		*	
<p>The Adeno-X qPCR Titration Kit is designed to be used with real-time PCR instruments to rapidly titrate adenoviral stocks from all Ad5-based adenoviral vectors. The protocol combines qPCR with green intercalating dye chemistry, allowing you to determine the viral genome copy number in adenoviral preparations (i.e., crude lysates or purified stocks) from a calibrated DNA standard curve. The kit includes a viral nucleic acid purification kit, a calibrated Adeno-X DNA Control Template, primers, and all the reagents required to conduct 200 TB Green-based qPCR reactions with either the DNA Control Template or purified viral DNA.</p> <p style="text-align: center;"></p> <div style="display: flex; justify-content: space-around; border: 1px solid #ccc; padding: 5px;"> Documents Components Image Data Resources </div>					

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