# Determination of adenovirus concentration using Biacore T200

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## Introduction

Virus particles have evolved the ability to introduce foreign nucleic acid into living cells, a property that makes them suitable as vectors to deliver genetic material. Viral vectors are being used in vaccine and therapeutic applications, such as gene therapy, cell therapy, oncolytic cancer, immunotherapies and tumor vaccines, and adenovirus constitutes the most widely used vector system in clinical trials. One of the most studied adenovirus vectors is the first generation of recombinant adenovirus serotype 5 (AdV5).

Analysis of virus particles and impurities to monitor the production process can be both time consuming and costly, and sometimes robustness and reproducibility may be difficult to achieve.

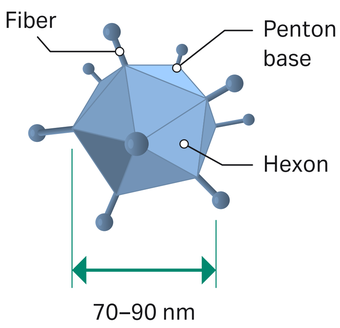
This poster focuses on the analytical steps in the development of an AdV5 production process, and describes the development of two assays for determination of adenovirus concentration using surface plasmon resonance (SPR). These assays are based on interactions with two separate proteins on the virus particle. By immobilizing recombinant human coxsackievirus and adenovirus receptor (CAR) protein or Factor X (FX) protein on a Biacore™ sensor chip and injecting virus particles, the adenovirus concentration were accurately assessed using Biacore T200. Using these approaches, two sensitive and robust assays were developed, showing strong correlation with standard adenovirus quantitation assays, such as real-time/quantitative polymerase chain reaction (qPCR). In comparison with qPCR, the described Biacore assays significantly reduced assay and hands-on time, thereby enabling their use in determination of virus content in critical steps of an adenovirus production process.


Biacore T200

## Background adenovirus

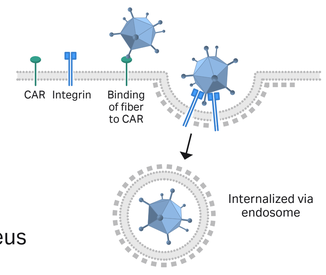
Adenovirus was isolated in 1953 and is among the most common viruses found in healthy people. More than 50 distinct adenoviral serotypes have been identified in humans. These serotypes can cause a wide range of conditions, from mild respiratory infections in children to more serious and life-threatening disease in people with weakened immune system.

Adenovirus virions are nonenveloped icosahedral particles and the capsid structure is made up of three proteins; fiber, penton base, and hexon. The virus is composed of around 1 million amino acid residues with a relative molecular mass ($M_r$) of 150 × $10^6$.



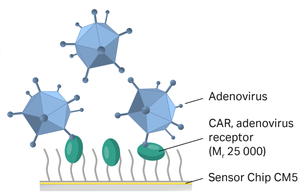

## CAR (coxsackievirus and adenovirus receptor) assay

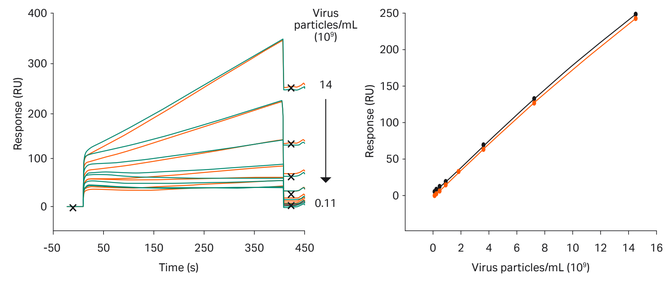
- CAR primary receptor on host cell
- Binding of fiber protein on virus particle to CAR
- Secondary binding of penton based protein to cell surface integrin receptors
- Uptake of virus via endocytosis
- Transport of viral genome into nucleus




### Experimental conditions

- Amine coupling of CAR to Sensor Chip CM5 (~ 2300 RU)
- Assay buffer: HBS-EP+
- Analyte contact time: 400 s at 5 µL/min
- Regeneration: 2 × 30 s Glycine pH 1.5
- Dilution factor: 100–200
- Calibration standard: ATTC (American Type Culture Collection)

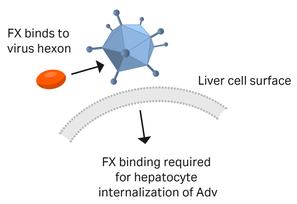



- Assay sensitive enough to allow for a high sample dilution factor thereby minimizing any matrix effects
- Good overlap of calibration curves run with 27 samples between first and second curve indicates great repeatability and assay stability
- < 5% CV of samples analyzed in duplicate

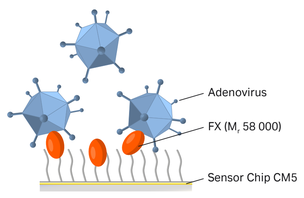
## FX (Factor X) assay

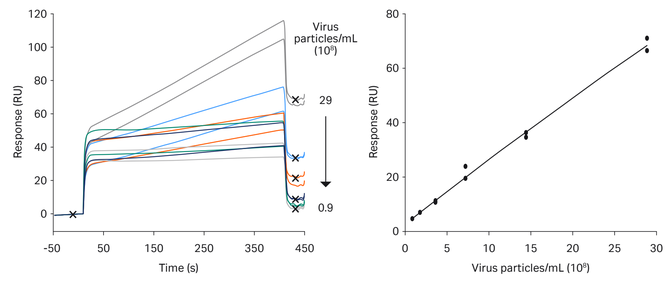
- Adenovirus dependent on coagulation factors to be able to infect liver cells
- FX binds to hexon protein on the virus particle
- Affinity of FX/hexon binding 40 times higher than the affinity of fiber binding to CAR
- FX binding is calcium dependent




### Experimental conditions

- Amine coupling of FX to Sensor Chip CM5 (~ 4000 RU)
- Assay buffer: HBS-P+, 5 mM $CaCl_2$
- Analyte contact time: 400 s at 5 µL/min
- Regeneration: 2 × 60 s HBS-EP+
- Dilution factor: 100–200
- Calibration standard: ATTC (American Type Culture Collection)

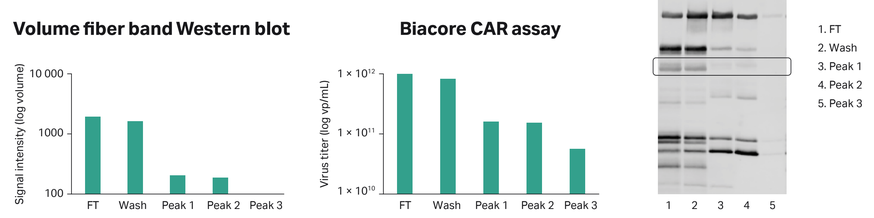



- Regeneration simplified through the use of HBS-EP+ buffer as regeneration solution
- Good repeatability and assay stability also for the FX assay indicated by overlapping calibration curves (27 samples run between first and second curve)
- ~ 5% CV of samples analyzed in duplicate

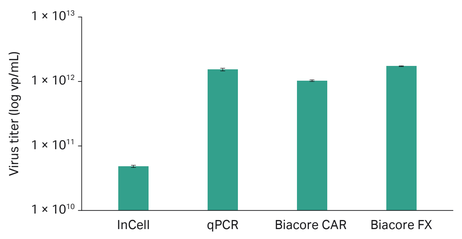
## Comparing Biacore CAR assay and Western blot

- Calculation of virus recovery in different fractions of the capture purification step
- Similar patterns for calculated adenovirus concentration
- Higher sensitivity for Biacore CAR assay – virus detected also in elution peak 3




## Comparing Biacore CAR and FX assays with qPCR and IN Cell Analyzer

- Calculation of adenovirus concentration in purified and concentrated bulk product
- Biacore CAR and FX assay reults were in good agreement with qPCR results
- IN Cell assay measures infectious titer while qPCR and Biacore assays measure total virus titer
- Infectious virus titer expected to be lower than total virus titer
- Regulatory requirements: ratio total/infections virus particles < 30




## References

1. Kalyuzhniy *et al.* Adenovirus serotype 5 hexon is critical for virus infection of hepatocytes *in vivo*. *PNAS* **105**, 5483–5488 (2008).
2. Data file: Biacore T200. Cytiva, KA7891280619DF 28979415AF (2019).
3. Application note: Determination of adenovirus concentration using Biacore T200. Cytiva, KA878080618AN (2018).
4. Application note: Downstream process development for efficient purification of adenovirus. Cytiva, KA876140818AN (2018).

## Conclusions

- The Biacore CAR and FX assays provide robust and reproducible results with lower CVs than established technologies
- Results correlate well with established methods, such as qPCR and Western blot
- The Biacore methods are less laborious than established methods with minimal assay and sample preparation and a higher degree of automation
- The assays can be used as analytical tools in optimization of process conditions through detection of fiber protein using Biacore CAR assay or detection of hexon using Biacore FX assay

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