# Titration of Adenoviral Vectors

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Version: 1.0 - Print Version (.PDF)

This protocol explains how to produce adenoviral vectors in a easy way. **Adenoviral vectors can be harmful!** Take care of the security issues and read more about it then only this protocol. I don't stress safety issues and basic things in this protocol, I expect that you know how to work with viruses and with tissue culture.

It is important to know the concentration of the functional adenoviral vectors. The concentration is measured in infectious units per ml stock solution.

To measure we split 293A cells in a 12 well in such way that they grew confluent on the next day.

On the next day we add to the confluent cells in 1 ml medium in the top row 100 µl, 30 µl, 10 µl and 3 µl of the adenoviral stock solution we want to test. Mix well. Pipet 10 µl of the top row to the well in the middle row below. Mix again well. These wells correspond to 1 µl, 0.3 µl, 0.1 µl and 0.03 µl

After 48 hours we can evaluate the result. Therefore we check in which well all the cells detached. We can shake the 12 well a little to promote it. In one well are $10^6$ cells. They detach after 48 hours if they were infected with 3-5 adenoviral vectors per cell (Multiplicity Of Infection, MOI 3-5). The amount that just allowed the cells to detach corresponds in our assay then to 5 x $10^6$ infectious units. Then you just calculate the amount back to one ml. This is not exact science. It has an error of the factor 2-3 and there are also differences between the persons doing the assay. To be more precise you can wait 36 hours and stain the cells with an anti-hexon antibody.

Small calculation help:
100 µl - 5 x $10^7$ IU/ml
30 µl - 1.7 x $10^8$ IU/ml
10 µl - 5 x $10^8$ IU/ml
3 µl - 1.7 x $10^9$ IU/ml
1 µl - 5 x $10^9$ IU/ml
0.3 µl - 1.7 x $10^{10}$ IU/ml
0.1 µl - 5 x $10^{10}$ IU/ml
0.03 µl - 1.7 x $10^{11}$ IU/ml

You can expect $10^8$ - $10^9$ IU/ml for tissue culture stocks and 10 - 50 times as much in animal stocks. Because of the high concentration you can start to titrate from 10 µl on if you test animal stocks.

## Known Issues:

- Too many freeze/thaw-cycles damage the adenoviral particles. Aliquot you stocks and store them at -80°C. Glycerol is not required.

## References and Comments:

I developed this protocol based on the instructions provided with the gateway vectors and the AdEasy protocol. I have done it many times. I guess my protocol gives a better overview, but have a look at the provided protocol from gateway for details and instructions were to buy the things.

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