Adenoviral System FAQ

What is the generation of the pAdeno vector?

The pAdeno system is a 2nd generation vector derived from human adenovirus type 5.

Which adenoviral vector is used for the construction of the pre-made Adeno-plasmids?

Human adenovirus 5

How is the titer determined to be 10^6 cfu/ml?

We produce the virus via an established procedure (standardize packaging mix and expression vector amount) that will give a minimal titer of 1X10^6 pfu/ml.

Does it require the purchase of additional products to produce adenovirus? If so what are the necessary related products?

Customers need 293 cells to amplify adenoviral constructs.

What is the required biosafety level for using recombinant adenovirus?

The recombinant adenoviruses are replication deficient due to deletions in the E1 and E3 regions. According to references issued by the NIH Office of Biosafety, recombinant human adenovirus has been classified in biosafety level II for agents considered of ordinary potential harm, and you need BL-2 level facility to work with it. It should be noted that cell culture facilities in most institutes are certified as BL-2 level. Wild type, replication competent adenoviruses could cause cold symptoms but generally do not cause serious illness. For more information on biosafety levels please visit http://bmbl.od.nih.gov

Our primary experimental model is the mouse. Will this system work for infecting primary mouse cells?

Most adenoviral vectors are human based adenovirus which will give 100% transduction efficiency in human cells except blood cells. Most mouse cells are also transducible with 100% efficiency with adenoviral vectors, but some of them are not transducible due to a lack of adenovirus receptors on their cell surface. A good way to solve this problem is to use a marker adenovirus like GFP to determine whether the cell posses the receptor or not before beginning a big project.

How do I check the efficiency of adenovirus infection in my target cells?

The Adenovirus has a very broad host range; it can infect human and other mammalian cell lines or primary cells, including dividing and non-dividing cells. There are in fact very few cell lines that cannot be infected. It has been shown that cells of Hematopoietic origin are more resistant to adenovirus infection, and so may need high quantities of viruses to achieve sufficient infection levels as those cells have only limited CAR receptors expressed on cell membrane.

For your convenience, we offer some marker adenoviruses, such as Ad-CMV-beta-Gal or Ad-GFP to allow you to evaluate infection efficiency in your target cells.

What's the optimal concentration of viruses for infection?

The appropriate MOI (multiplicity of infection) of recombinant viruses used for infecting cells is very important for the outcome of your experiments. If MOI is low, it will not give 100% of infection. If MOI is too high, it will cause cytotoxicity or other undesired effects. You should use the minimal virus concentration that will give 100% gene delivery. This optimal concentration differs dramatically among different cell types. To determine optimal concentration of virus, you could perform pilot experiments by using marker adenoviruses, such as Ad-beta-Gal or AdEGFP. For most cell types, a viral concentration of of 2 x 10e5- 1 x 10e6 per ml of media gives 100% of infection without visible side effects. For your reference, we recommend the following amount virus-containing media for infection (given that the adenovirus concentration is 1 x 10e6 particles per ml):

10-cm plate: 5 ml per plate
6-well plate: 1 ml per well
12-well plate: 0.5 ml per well
24-well plate: 0.2 ml per well
This volume roughly covers the surface area of each well or plate.

For *in vitro* use (cell culture studies), is viral purification required?

No. If viruses will be used in *in vitro* cell cultures, double CsCl purification is not required. For *in vivo* studies (i.e. animal studies), purification is essential in order to remove defective particles, cell debris, and small amounts of media components, since these contaminants induce significant immune responses. In addition, CsCl purification will concentrate the virus to a level suitable for *in vivo* injections.

What are the recommended storage conditions of recombinant Adenoviruses?

For long-term storage, the virus should be kept at -80C, especially after CsCl or chromatography purification. At -80C in 50 mM Tris (pH 7.0) plus 5% glycerol, the viruses could be stable for several years.

Alternatively, viral stock could be stored in 10mM Tris (pH 8.0) plus 4% sucrose for in vivo injection as it is more difficult to do in vivo injection with glycerol formulation.

What are RCAs?

One concern when working with adenoviral vectors is the rare occurrence of replication competent adenoviruses (RCAs) in a population of replication-deficient viruses. RCAs can emerge as a result of the rare double crossover through overlapping sequences present in the recombinant adenovirus and the genome of 293 cells. This event results in the replacement of the transgene by E1 region. Once this happens, the adenovirus could replicate in target cells (non-permissive cells). To detect RCA, non-permissive cells, such as A549 cells, are incubated with the viral stocks and monitored for cytopathic effects (CPE). According to NIH guideline, <1 plaque in about 10^4 viruses is considered safe to use in clinical trials. To avoid the occurrence of RCA, viruses should be produced and amplified in low passage 293 cells.

What is the concentration or titer of the premade adenovirus?

The seed stock is 10^6 pfu/ml; however, the customer can amplify as much as needed in 293 cells.

What are viral particle (VP), plaque formation unit (PFU), and infectious unit (IFU)?

Viral particles (VPs) represent the total number of viral particles (infectious and infection-deficient combined). Due to variations in virus preparations, the ratio of infectious /non-infectious varies significantly and therefore, VP does not reflect the concentration of virus in a preparation. PFU (plaque forming unit) represents the number of infectious or live viruses. It reflects the concentration of infectious viruses in a preparation. IFU (infectious unit) is biologically equivalent to PFU. For most virus preps, the VP/PFU ratio is 20:1 to 50:1.

What are some methods to determine adenovirus titers?

There are 3 commonly used protocols for determining adenovirus titer: (1) OD260 Assay, (2) Plaque Formation Assay, and (3) End-point Dilution Assay.

OD260 assay measures the concentration of viral DNA. It does not distinguish intact, infectious viruses from damaged, non-infectious ones. It is a physical assay measuring the number of total viruses, live and dead. Based on OD260, the concentration of viral particles (VP) could be obtained. To measure the OD260, the virus stock has to be purified first.

On the other hand, plaque formation assay measures the concentration of infectious viruses, and therefore it is a biological assay. Basically, a monolayer of 293 cells is infected with a series of virus dilutions from

10^1 to 10^12. Viruses will propagate in infected cells, and eventually cause complete cytotoxicity effects (CPE) in that cell, and get released. The released viruses will infect neighboring cells, and the whole process will be repeated, eventually leading to the formation of holes or plaques on the cell monolayer. In order to prevent the diffusion of viruses among plaques, a layer of agarose is laid on top of cells after initial infection.
The biological principle or End-point Dilution assay is similar to the plaque formation assay, although the procedure and measurement is different, and the formula for calculating the virus titer is a bit more complicated.

Although both the Plaque Formation Assay and End-point Dilution assay gives the titer of infectious or working viruses, they are scored by human eyes and subjected to human and procedure variations. For the same virus stock, it is quite common that different people will get significantly different titer readings.

How does $TCID_{50}$ work?

Please see the link before for the assay. TCID50 is the same as end dilution assay.
http://www.abmgood.com/TechSupport/adeno-vec.php

If the customer provide us the virus, we can provide its titer with the Cat#C008 service.

Does the order come with a control or empty virus?

The control or empty vector virus (Adeno CMV-null) is sold separately. You can find this product under the Cat. No. 000047A.

What is the cell density that the customer should use for adenovirus infection?

70%

Is it possible to use the adenovirus directly without amplification in 293 cells? Can we use other cells than 293?

Adenoviruses are supplied as a seed stock of 1x10^6 pfu/ml, and should be amplified before use. If not enough virus is used, it will not give 100% of infection. The Adenovirus has a very broad host range; it can infect human and other mammalian cell lines or primary cells. If you find that your cell line may be more resistant to Adenovirus infection, you may need to use high quantities of the virus to achieve sufficient infection levels.

What cells do I use to amplify the adenovirus?

It must be 293 cells for amplification. 293T cells will not work as well.

Are the His and HA tags placed at the N or C terminus?

All adenovirus His and HA tags are at the C-terminus (unless otherwise specified).

Do you have more information about the insert sequence?

The insert contains the full CDS region of the gene according to the accession no. listed above.

I have received more than 250ul of seed stock and have concerns it has been diluted or is the wrong product entirely.

We occasionally have an excess from production of our adenoviruses and in these cases we choose to distribute it as an additional volume to the stated 250ul for our customer's benefit. Please be assured you have received the correct item, the viral titer will still be 10^6 pfu/ml

I just received 10^6 pfu/ml Adenovirus stock from your company, and amplified according to the protocol provided. What is the estimated final PFU after 3 cycles of freezing-thawing?

The neat supernatant from amplifying the virus will still be 10^6pfu/ml. The virus will need to be concentrated if a higher titer is required.

If you wish to achieve a higher titer, we recommend using one of our purification kits:
http://www.abmgood.com/Adenovirus-Purification.html

How do you determine the viral titer of this product?

The viral titer will be calculated by end-point dilution assay. Any additional assays required (Plaque Assay, RCA Assay or TCID50 Assay) can be provided on request, at an additional cost.

What is an easy to transduce cell line for adenoviruses?

293 can be used to check expression, however, transduction will lead to plaque formation therefore a better cell line is MCF7, which are easily transduced by adenovirus and is not affected by adenovirus CPE.

If my cells don't divide, would the adenovirus still be considered to confer transient effects?

Yes, even if your cells do not divide, the adenovirus would still be able to infect your cells.

What type of media is recommended for adenovirus amplification?

We recommend standard DMEM + 10% FBS (abm Cat#TM999), and 1% P/S (abm Cat#G255)

For the amplification protocol, what volume of media is recommended for a 60mm dish or 100mm dish?

We recommend ~3-4ml per 60mm dish and ~10ml per 100mm dish.