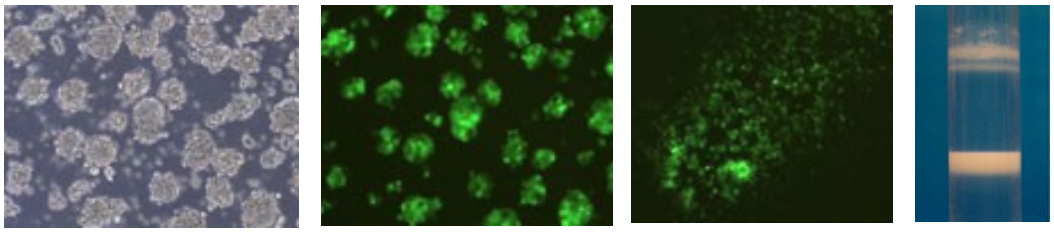
GeneCust
Custom Services for Research

Adenovirus and Lentiviral Services

GeneCust Europe



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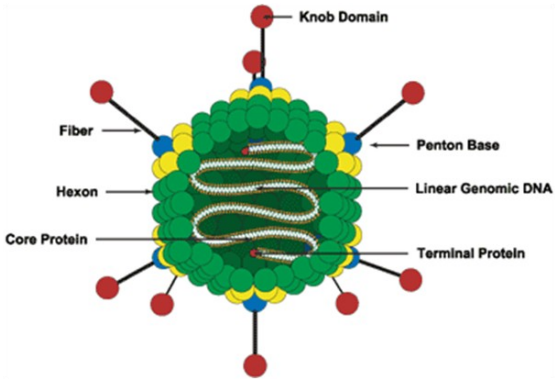
Viral Services

Adenovirus Services

- Adenoviruses are double-stranded DNA viruses that can cause a number of disorders, such as respiration infections. The virion is non-enveloped, spherical and about seventy to ninety nm in size. The adenovirus DNA is linear, double stranded of approximately 36,000 bp wrapped in a histone-like protein and has inverted terminal repeats (ITRs) of 50-200 bp. Both strands of adenovirus DNA encode genes and the genome encodes about thirty proteins.



Soon after its isolation in 1953 adenovirus was recognized as a preferred vehicle for gene delivery because of its many distinguishing features. The first generation recombinant adenoviruses are E1 deleted to insure replication deficiency of the virus and to prevent cell lysis. Once packaged into a complementing cell line (a cell line that provides the E1 products in trans, e.g. HEK 293A cells), viral replication will be enabled. In addition to being E1 deleted, the first generation adenoviruses are also often E3 deleted (E1/E3).

Adenovirus Services

Adenovirus vectors are arguably the most widely used gene deliver system. GeneCust has been providing related services to researchers in academic institutes, pharmaceutical and biotech industrials for the past five years and have constructed, amplified, and purified many adenovirus. GeneCust's service has enable researchers to save time and money in their works. Currently, GeneCust is providing a complete line of services relating adenovirus vectors:

Catalogue #	Description	Timeline	Price
S1100	Construct an adenovirus vector to express a gene : GeneCust will use insert your sequence into the adenoviral genome and return a crude cell lysate containing the viral construct (approximately 10^9 viral particles/ml) within 3 weeks. The crude cell lysate is suitable for testing to ensure that the virus performs as expected. Additional amplification and purification of the virus **(Cat#S3000)** will require another 2 weeks. Typically we will need about 10 ug of a plasmid containing the gene to be expressed.	3-4weeks	1000 €
S1200	Construct an adenovirus vector to express a gene (target cDNA not provided by customer) : If a customer does not provide the cDNA, GeneCust will clone the cDNA or purchase the cDNA for the customer. The cDNA will be inserted into our adenovirus system and generate adenovirus. A crude cell lysate containing the viral construct (approximately 106 viral particles/ml) can be delivered to the customer within 3 weeks. The crude cell lysate is suitable for testing to ensure that the virus performs as expected. Additional amplification and purification of the virus (Cat# S3000) will require another 2 weeks.	4-5 weeks	quote
S1300	Generation of adenovirus (adenoviral construct provided) : By using this service, the customer provides the adenovirus construct and GeneCust will generate adenovirus in 293 cells. GeneCust will deliver a crude cell lysate containing the viral construct (approximately 106 viral particles/ml) within 3 weeks. The crude cell lysate is suitable for testing to ensure that the virus performs as expected. Additional amplification and purification of the virus (Cat#S3000)will require another 2 weeks.	3 weeks	1000 €

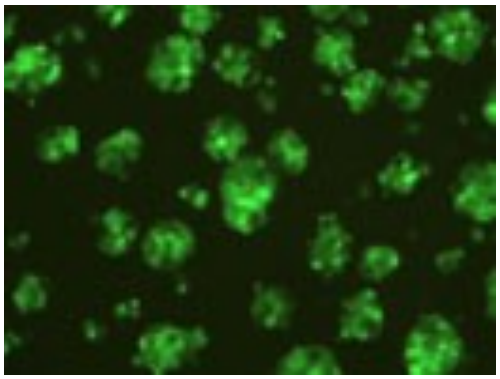
Catalogue #	Description	Timeline	Price
S2100	Construct a pQuiet vector to silence gene expression : The customer will provide us a validated shRNA (short hairpin RNAi) sequence. GeneCust will synthesize a double-stranded oligonucleotide that encodes the validated shRNA sequence and inserted into a pQuiet vector. The vector will be amplified and sequenced to ensure that the correct shRNA sequence will be expressed. You will receive your plasmid within 3 weeks. If you would like more than one sequence to be inserted into pQuiet 2, 3, or 4 for multiple gene silencing, the additional cost will be charged per sequence. All you need to do is to provide the validated, effective 19 bp sequences from the target genes.	3 weeks	1000 €
S2200	Construct an adenovirus to silence gene expression : The customer will provide GeneCust an effective shRNA sequence. GeneCust will be synthesized and inserted it into pQuietU6 vector and convert it into an adenovirus. A crude cell lysate (106 vp/ml) can be shipped to customer within 3 weeks. The crude cell lysate is suitable for testing to ensure that the virus performs as expected. Additional amplification and purification of the virus (Cat#S3000) will require another 2 weeks.	3 weeks	1000 €
S2300	RNAi selection service : Three short hairpin RNA (shRNA) sequences will be selected and cloned into a pQuietU6 plasmid. The desired constructs will be sequenced to verify the correct shRNA. The 3 shRNA plasmids will then be co-transfected into 293 cells with an cDNA expression plasmid. The most effective shRNA can be determined by a Western blot. GeneCust guarantees that the selected vector will provide 70% silencing efficiency in a co-transfection experiment.	4 weeks	1000 €
S3000	Amplification and purification of adenovirus (3 ml) : Existing adenovirus vectors supplied by our customers or newly constructed vectors will be amplified to obtain more than 3 x 10exp12 viral particles, which requires approximately 2 weeks. The adenovirus will then be purified by centrifugation using two sequential cesium chloride gradients. The final product (3 ml, 1x10exp12 viral particles/ml) will be dialyzed, tittered spectrophotometrically, and tested for sterility, and then shipped to customer.	2 weeks	1000 €
S3010	Amplification and purification of adenovirus (1 ml) : GeneCust provides a customer 3 ml of 1x1012 viral particles/ml in our standard amplification/purification services (CAT#3000). If a customer needs less amount of adenoviral stock, GeneCust also can do it for you. Existing adenovirus vectors supplied by our customers or newly constructed vectors will be amplified to 1 x 10exp12 viral particles. The adenovirus will then be purified by centrifugation using two sequential cesium chloride gradients. The final product (1 ml, 1x10exp12 viral particles/ml) will be dialyzed, tittered spectrophotometrically, and tested for sterility, and then shipped to customer. The service time is approximately 2 weeks.	2 weeks	800 €
S3110	Plaque purification : Newly generated vectors that have been constructed using our adenoviral system do not require plaque purification, but when previously amplified vectors are re-amplified, rare crossover events can result in the appearance of RCA (Replication-Competent Adenovirus), which must then be eliminated by plaque purification. Recombination is expected to occur only after adenovirus has been amplified extensively and generally results in replacement of the desired transgene with the E1 gene that was deleted to make the virus replication-deficient. Amplification of viral stocks contaminated by RCA results in overgrowth by the RCA. It is always prudent to perform an RCA assay (#S3130 or S3140) before re-amplifying a viral stock.	2 weeks	1000 €

Catalogue #	Description	Timeline	Price
S3120	Plaque assay to titer adenovirus : The abundance of adenoviral particles can be determined in a variety of ways. Two of the most useful are the spectrophotometric method, which is fast and highly reproducible, and the plaque assay, which estimates the number of infectious particles that are present. The plaque assay is performed by treating host cells in which the virus can replicate with serial dilutions of the virus preparation for several days. During the incubation, infected cells grow new viral particles that infect neighboring cells, which die and release more adenovirus until a plaque becomes visible. The plaque assay is slower and less reproducible than the spectrophotometric assay, but it provides a result that may be a more realistic estimate of the number of viral particles needed to evoke a biological response. A normal ratio between the spectrophotometric and plaque assay results indicates that a purified viral product is not contaminated with UV absorbent material.	1-2 weeks	350 €
S3130	PCR assay for RCA : The DNA purified from viral particles is used as a template for a PCR procedure in which E1 gene specific primers are used to detect the presence of the E1 gene. The sensitivity of this method is limited by the amount of template DNA that can be added. Greater sensitivity (10-fold) can be achieved by doing 10 replicate PCR reactions. The PCR procedure to detect the presence of RCA is faster and more sensitive than the assay that employs A549 cells and is recommended before amplification of a viral stock.	3 days	400 €
S3140	RCA assay in A549 cells : The standard method to determine the presence of RCA (Replication-Competent Adenovirus) is to inoculate A549 cells with a sample of the viral stock. Since A549 cells are very readily infected by adenovirus but do not express the E1 gene, no plaque will form unless viral particles that have acquired the E1 gene are present. The sensitivity of this method is limited by the cytotoxic effects of the replication-deficient virus on the A549 cells, which limits the number of particles that can be added to the cultured cells. Approximately 1 RCA per 106 viral particles (determined spectrophotometrically) can be detected by this method.	1 week	400 €
S3150	TCID50 : HEK293 cells growing on 96 well plates will be infected with serial dilutions of an adenoviral vector stock and the resulting infection pattern will be evalulated to determine the TCID50 (Tissue Culture Infectious Dose 50) using the Karber formula.	1-2 weeks	800 €
S3160	Isolate adenoviral DNA sample : More than 5 ug high quality adenoviral DNA will be purified from approximately 1011 viral particles.	1 week	500 €
S3170	MOI Assay : The multiplicity of infection or MOI is the ratio defined by the number of infectious virus particles divided by the number of target cells. In this service, MOI will be assayed using cells (provided by customer or by GeneCust). It usually takes two to three weeks.	3 weeks	quote

Premade Adenovirus

Many premade adevovirus are available as experiments controls :

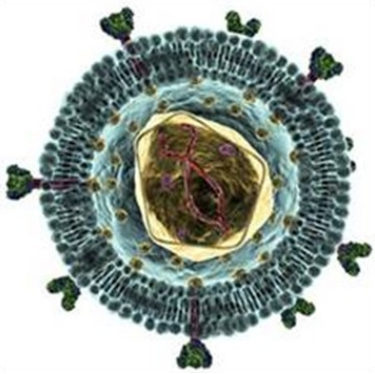
Ad-CMV-Null
Ad- ß-gal/lacZ
Ad-GFP
Ad-Luciferase
Ad-scramble-shRNA
Ad-Scramble-shRNA with GFP
Ad- ß-gal-shRNA
Ad-GFP-shRNA
Ad-Luciferase-shRNA
Ad-U6 Null



Price for 1 ml = 10exp12 VP : 400 euros/ml
Price for 3 ml = 3 x 10exp12 VP : 350 euros/ml
Pirce for 5 ml = 5 x 10exp12VP : 300 euros/ml

Lentivirus Services

Lentiviruses are a subclass of retroviruses. The viral genome in the form of RNA is reverse-transcribed when the virus enters the cell to produce DNA, which is then inserted into the genome at a random position by the viral integrase enzyme. Lentiviruses have been adapted as vectors thanks to their ability integrate into the genome of non-dividing as well as dividing cells. The vector can be used to provide highly effective gene therapy as lentiviruses can change the expression of their target cell's gene for up to six months. They can be used for nondividing or terminally differentiated cells such as neurons, macrophages, hematopoietic stem cells, retinal photoreceptors, and muscle and liver cells, cell types for which previous gene therapy methods could not be used. The only cells lentiviruses cannot gain access to are quiescent cells (in the G0 state) because this blocks the reverse transcription step.



For safety reasons lentiviral vectors never carry the genes required for their replication. Lentiviral vectors are usually created in a transient transfection system in which a cell line is transfected with three separate plasmid expression systems. These include the transfer vector plasmid (portions of the HIV provirus), the packaging plasmid or construct, and a plasmid with the heterologous envelop gene (ENV) of a different virus. The three-plasmid components of the vector are put into a packaging cell which is then inserted into the HIV shell. The virus portions of the vector contain insert sequences so that the virus cannot replicate inside the cell system.

Delivery of genes by lentiviral vectors has been well established and is currently widely used due to several advantages: Lentiviral vectors can accommodate long sequences; the vectors seem to be non-immunogenic due to the lack of viral coding sequences transfer; the vectors are able to transduce non-dividing cells; and the products can be stably expressed due to integration into the cell chromosome.

Catalogue #	Description	Timeline	Price
SL1000	Mini-scale Lentivirus Production : This service includes: Customer submits 10 ug DNA prepared by Qiagen Kit GeneCust delivers 2-4 ml of virus Concentrated viral stock is not available for this service	3 weeks	800 €
SL1100	Standard Lentivirus Production : For this service: Customer submits 100 ug DNA prepared by Qiagen Kit GeneCust delivers 100 ul of concentrated viral stocks (approximately 1 x 10exp11-12 LP/ml)	4 weeks	1200 €
SL1200	Large-scale Lentivirus Production : Customer submits 3 mg DNA prepared by Qiagen kit GeneCust delivers 1 ml of concentrated viral stocks (approximately 1 x 10exp11-12 LP/ml)	6 weeks	10000 €
SL2000	Customer Lentiviral Construction and Production : The service includes: Customer provides the lentviral vectors and cDNA. GeneCust will clone the cDNA into the lentiviral vectors GeneCust produces high titer of lentviirus (100 ul concentrated viral stocks, approximately 1 x 10exp11-12 LP/ml)	5 weeks	2000 €
SL3000	Customer Lentiviral RNAi Services : The service includes: RNAi design, selection, validation Guarantee 70% silencing effect in a cotransfection experiment Lentivirus construction and production Delivery of 100 ul of concentrated viral stocks (approximately 1 x 10exp8-9 TU/ml)	7-8 weeks	3500 €
SL4000	Lentivirus Titering Assay : In this service, the titer of lentivirus (provided by customer or constructed by GeneCust) is determined using 293 cells. The assay is usually takes 2 weeks to finish.	1 week	500€

FAQS

Q. What is the required biosafety level for handling recombinant adenovirus?

A. Our recombinant adenoviruses that are deleted in the E1 and E3 regions will replicate in HEK293 cells, but not in other cell lines. According to 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.
For more information on biosafety levels, please refer to the following CDC publication: Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, May 1999; this publication is also available at http://bmbl.od.nih.gov.

Q. What type of cells are used to produce and grow recombinant Adenoviruses in your systems?

A. Human Embryonic Kidney cell line, the HEK-293 cells. The 293 cells contain the full E1 region of the Adenovirus type 5, from nucleotides 1 to 4355 of Ad5 wt, making these cells suitable for the generation and growth of helper-independent recombinant Adenoviruses.

Q. How are recombinant adenoviruses purified and virus titers determined?

A. The amplified recombinant adenovirus was purified on 2 sequential cesium chloride gradients and then dialyzed with a buffer (PBS, 10% glycerol, pH7.4) to reduce the salt concentration. The titer of purified virus can be determined with (1) OD260 Assay or (2) Plaque Assay.

The OD260 assay measures the concentration of viral DNA and protein. It does not distinguish between intact, infectious viruses and damaged, non-infectious viruses. It is a physical assay measuring the concentration of total viruses, live and dead. Based on OD260 data, the concentration of viral particles (VP) can be calculated using the following formula:

Adenovirus titer in viral particles/ml = OD value x *d* x 1.1 x 10^{12}; (*d*= dilution factor)

The plaque assay measures the concentration of infectious viruses, and therefore it is a biological assay. Basically, a mono-layer of HEK293 cells are infected with a series of virus dilutions. Viruses will propagate in infected cells, and eventually leading to the formation of holes or plaques. Thus, a PFU can be determined from a plaque assay.

Q. What are the differences between viral particle (VP) and plaque formation unit (PFU)?

A. Viral particles (VPs) represent the total number of viral particles (live and dead combined). Because of variations in viral preparations the ratio of live/dead varies significantly. Thus, VP does not reflect the amount of live virus in the preparation.

PFU (plaque formation unit) represents the number of infectious or live viruses. It reflects the amount of working viruses in the preparation.

For most virus preps, the VP/PFU ratio is 20:1 to 50:1.

Q. What are the conditions recommended for the storage of recombinant adenovirus preparations?

A. For long-term storage, the virus should be stored at -80°C, especially after CsCl purification. At -80°C, the virus could be stable for at least a year. However, the repeated freeze-and-thaw should be avoided, since it will cause significant decrease of titer.

FAQS

Q. What size of insert can be cloned into the Adenoviral expression system?

A. The insert size limitation for the DE1/E3 deleted Ad5 vector is about 8 Kb in length.

Q. What are RCAs?

A. The replication competent adenoviruses (RCAs) is a result of the rare double crossover through overlapping sequences present in the recombinant adenovirus and the genome of HEK293 cells. This event results in the replacement of the transgene by E1 region. Once this happens, the adenovirus could replicate, without the need of a complementing cell line. Usually two methods, non-complementary A549 cells or PCR could be used to detect RCA in a recombinant adenoviral preparation.

According to NIH guideline, <1 plaque in about 1E4 viruses is considered safe to use. To avoid the occurrence of RCA, viruses should be produced and amplified in low passage packaging cells.

Q. What MOI (multiplicity of infection) should I use with my cells?

A. Usually, a MOI of 1 is suitable to infect 293 cells. If you use other cell lines and the susceptibility to Adenovirus infection is unknown, you should test a MOI (number of virus per cell) range between 1 and 1000. The MOI may be increased up to 10000 when testing a mouse cell line. For most cell lines, the transfer of a reporter gene (for example, GFP) to 100% of cells, without any signs of toxicity, can be achieved with a MOI of 10-100.

Q. Which viral expression system (adenovirus, retrovirus and lentivirus) should I use for my experiments?

A. Adenovirus can infect many types of mammalian including dividing and non-dividing or primary cells with high efficiency. They do not disrupt the genome of the host cell but remain in the nucleus as episomal DNA. However, adenovirus only provides a transient, high level gene expression in vitro and in vivo.

Retrovirus has low infection efficiency (<30%) in most cell types, and requires active cell division. In addition, there is a significant risk of integration into the host genome, leading to mutation of genes or activation of onco-genes in the host system. Unlike adenovirus, the costs of production of recombinant retrovirus are very expensive, which may limit your in vivo application.

Like Retrovirus, Lentivirus has low infection efficiency (<30%) in both dividing and non-dividing cells. Lentivirus also can integrate into the host genome, leading to mutation of genes or activation of oncogenes in the host system. As with retrovirus, the costs of production of recombinant retrovirus are very expensive.

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Thank you for your time.

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