GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Email: orders@gentarget.com
Fax: 1 (800) 380-4198

FAQ for pre-made Adenovirus:

1. How do I use the GenTarget's pre-made Adenovirus?

Pre-made Adenovirus is provided ready to us. Simply add it into the mammalian cell line of your choice. The amount of virus to add is dependent upon your cell types, or your titer the virus amount based on MOI number.

2. What is MOI?

To obtain the desired expression level, you need a suitable multiplicity of infection (MOI). The MOI is defined as the number of virus particles per cell, and a range of MOIs, from 0, to 1000 should be tested to determine the MOI to use. Normally, actively dividing cell require less virus (a lower MOI) than non-dividing cells. An MOI that is too high may lead to cell death due the toxicity of the virus or of the expressed target; an MOI that is too low may result in a low positive transduction rate and low target expression levels. You should use the minimal MOI required to produce the desired expression level or positive transduction rate (usually 100%). For most cell lines an MOI of 1-10 is fine.

3. What is the control virus?

The **AVP-Null** control virus (sold as a separate product) serves as the negative control for adenovirus treatment. The Null-control adenovirus is produced from an Adenovector cloned with a Null sequence which does not express any target.

4. How do I now the Adenovirus is working in my cells?

Adenovirus can transduce the majority of mammalian cells very well -- including human, mouse, and other species--whether dividing or non-dividing. However, there are a few cell types that cannot be transduced or that can be transduced only with very low efficiency. For those types of cells, you may wish to try lentivirus. The best way to find out if you cells are susceptible to adenoviral transduction is to use a fluorescent control adenovirus, which will let you easily visualize transduction as fluorescence signal. GenTarget provides pre-made adenoviruses, expressing a wide selection of fluorescent makers including GFP, RFP, YFP, and CFP, as well as fluorescent fusion markers.

5. How long will target expression last?

Typically, target expression can be detected after 12-24 hours. Depending upon the cell types or dividing cycles, the expression peaks at 2-3 days post

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Email: orders@gentarget.com
Fax: 1 (800) 380-4198

-transduction and persists for as long as the viral genome is present, from 1 week to 6 months or longer.

6. Can pre-made adenovirus be used *in vivo*?

Yes GenTarget provides adenovirus in PBS with 5% sucrose for *in vivo* applications. **NOTE:** all GenTarget pre-made Adenoviruses are **for research use only and not for clinical or therapeutic applications.**

7. What buffer is the virus provided in?

GenTarget's adenoviruses are provided as 200 µl aliquots in two formats:

- Crude viral stock in DMEM medium with 10% serum
- Concentrated adenovirus re-suspended into PBS with 5% sucrose for *in vivo* use.

8.What about bio-safety? What is RCA?

Adenoviruses are safe to use. They are non-replicable viral particles produced from an expression vector derived from human adenovirus type 5 genome in which the entire E1 (4.3kb) and E3 region was removed. E1 protein is essential for the viral replication without it, the packaged adenovirus cannot be replicated in target cells . The presence of the viral genome is transient since it will eventually be diluted out as cell division occurs. (Note: In E1 complementing cell lines, like 293A cells, the adenovirus can be replicated or amplified.)

Despite the adopted safety features, recombinant human adenovirus has been classified as a biosafety level II agent, and you will need a BL-2level facility to work with it. It should be noted that cell culture facilities in most institutions are certified as BL-2 level. Please refer to CDC and NIH guidelines for more details about adenovirus handling.

In rare instances, the E1 sequence from the genome of the virus producing (293A) cells is integrated into and adenoviral trans-gene construct by sequence homologous recombination, resulting in a (**RCA**). In theory, this would most likely occur in large-scale virus amplification. Wild type, replication competent adenoviruses could cause cold symptoms but generally do not cause serious illness. All GenTarget adenoviruses are made on a very small scale and RCA testing is not routinely performed for pre-made adenovirus. When performing large scale adenovirus applications, we use PCR to measure the level of replication competent adenovirus. **Note:** When RCA occurs, it will quickly overtake the non-replicable virus and cause

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Email: orders@gentarget.com
Fax: 1 (800) 380-4198

cell death. To avoid the occurrence of RCA, viruses should be produced and amplified in low passage 293A cells.

9. How is viral titer measured??

The titer (IFU/ml) of GenTarget's adenovirus is measured via a fluorescent marker (GFP or RFP) after transduction of the virus into HeLa cells. This titer approximates the real infectious units measured by biological (plaque) assay, and is used as the reference titer for the non-fluorescent construct.

10. How are the adenovirus vectors constructed?

GenTarget uses its proprietary Eco cloning technology (vector built-in cloning competent cells) to directly insert a target into the E1E3 deleted human adenovirus 5 genome.

11. Are there any antibiotic markers included in the adenovirus constructs?

Adenovirus is not integrated into the host cell genome therefore, it is not stable for long term expression and we do not include an antibiotic marker in adenovirus constructs. If some cases, we d=include a fluorescent marker under the same promoter for the specific target, mediated by F2A element. The fluorescent marker enables monitoring of viral performance and target expression, as well as selection via fluorescent signal. For long term stable expression, please use our pre-made lentivirus r which features a wide variety of selection markers.

12. Adenovirus vs. Lentivirus

Both Adenovirus and lentivirus can be transduced into dividing and non-dividing cells, and both are used as expression delivery tools for mammalian cell lines or primary cells. Unlike lentivirus, adenovirus is non-insertional so constructs do not integrate into the host genome and will not affect the activity of host genes. Adenovirus is a transient expression delivery vehicle; once transduced into mammalian cells, the recombinant adenoviral trans-gene target will be expressed until the viral genome is diluted by cell division. Trans-gene expression decreases gradually in actively dividing cells(normally in 1-2 weeks) but expression can persist for a longer time in non-dividing or slowly dividing cells, such as skeletal muscle cells or neurons. By contrast, lentivirus delivers stable, long term expression. Lentivirus also is less immunogenic to human cells and less toxic. Adenovirus may, however, have better transduction efficiency in some cell types.

GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Email: orders@gentarget.com
Fax: 1 (800) 380-4198

Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
ImmunoOncology Research	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	**CARs** Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
Infectious Antigens	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.

GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Email: orders@gentarget.com
Fax: 1 (800) 380-4198

Product Category	Product Description (please click into each category's page)
shRNA Knockdown	Knockdown verifeid and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla, Red-Luc and more,** with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.

GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Email: orders@gentarget.com
Fax: 1 (800) 380-4198

Product Category	Product Description (please click into each category's page)
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.