Pre-made expression Adenovirus product manual

Catalog#	Product Name	Amounts
AVP001	**RFP** adenovirus	1x10^{9} IFU/ml x 200ul in DEME with 10% FBS
AVP002	**CFP** adenovirus	
AVP004	**Luciferase / GFP** adenovirus	
AVP005	**Luciferase / RFP** adenovirus	
AVP011	**GFP** adenovirus	
AVP012	**YFP** adenovirus	
AVP017	**BFP** adenovirus	
AVP010	CRISPR / **hCas9** Adenovirus	
AVP013	h **OCT4** expression Adenovirus	
AVP014	h **SOX2** expression Adenovirus	
AVP015	h **LIN28** expression Adenovirus	
AVP016	m **MKOS** chained iPSC Adenovirus	
AVP-Null	**CMV-Null control** adenovirus	
AVP001-PBS	**RFP** adenovirus, in vivo ready	1x10^{11} IFU/ml x 200ul in PBS with 5% sucrose
AVP002-PBS	**CFP** adenovirus, in vivo ready	
AVP011-PBS	**GFP** adenovirus, in vivo ready	
AVP012-PBS	**YFP** adenovirus, in vivo ready	
AVP004-PBS	**Luciferase / GFP** adenovirus, in vivo ready	
AVP005-PBS	**Luciferase / RFP** adenovirus, in vivo ready	
AVP017-PBS	**BFP** adenovirus, in vivo ready	
AVP010-PBS	CRISPR / **hCas9** Adenovirus, in vivo ready	

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AVP013-PBS	h **OCT4** expression Adenovirus, in vivo ready	
AVP014-PBS	h **SOX2** expression Adenovirus, in vivo ready	
AVP015-PBS	h **LIN28** expression Adenovirus, in vivo ready	
AVP016-PBS	m **MKOS** chained iPSC Adenovirus, in vivo ready	
AVP-Null-PBS	**CMV-Null control** adenovirus, in vivo ready	

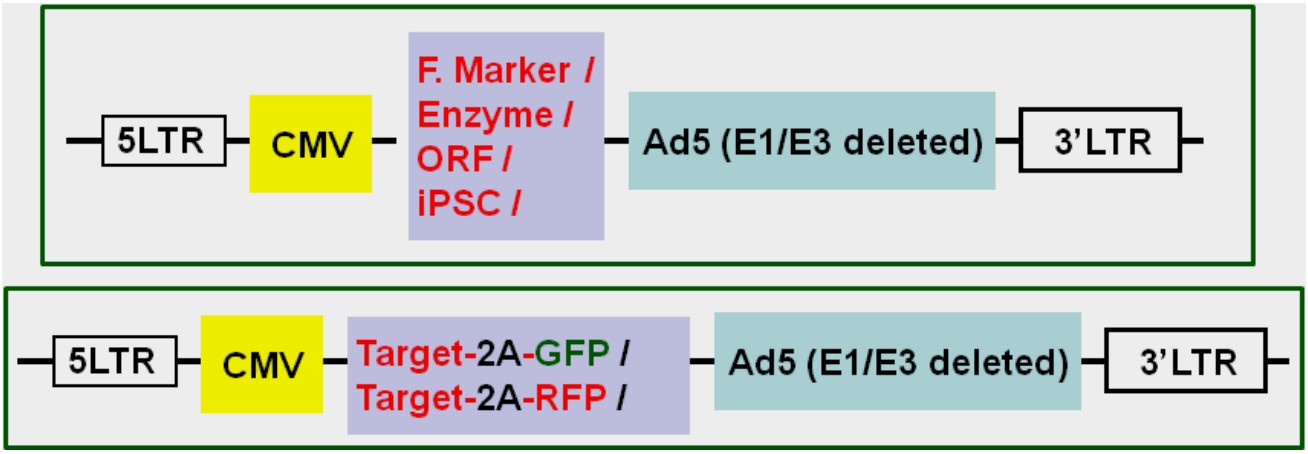
Storage: Store adenovirus at **<** -70°C, avoid repeat freeze/thaw cycles. Stable for 12 months.

Product Description:

Adenoviruses are double-stranded DNA viruses that can infect a broad range of cell types including dividing and non-dividing cells, and therefore are frequently used for gene delivery. Our Adenovirus expression vector is derived from human adenovirus type 5 with deletions of the E1 and E3 genomic regions. Because E1 is essential for the assembly of virus particles, our adenovirus system only produces replication-incompetent adenovirus. Viral replication can occur only in cell lines, such as 293A cell, that provide the E1 region.

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 target transgene will be expressed until cell division dilutes the viral genome. Transgene 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.

Our pre-made expression adenovirus contains a target expressed under an enhanced human cytomegalovirus (**suCMV**) promoter. When applicable, a fluorescent marker (**GFP or RFP**) is bicistronically expressed under the same promoter mediated by a 2A element, which allows expression of two individual targets. Please see the vector map below for the core expression cassette.



Note: We also provide adenovirus services for cloning your gene of interest and generates ready-to-use adenovirus particles with the best prices and fastest turnaround time.

Key features:

1. **Pre-made, ready-to-use**: No need for cloning or virus production. Simply add the virus to your cell line for target expression.
2. **Safe to use**: our adenovirus is produced from a vector with E1 and E3 deletions resulting in replication-incompetent virus.
3. **High expression level**: the target is driven by an extremely strong suCMV promoter. Virus titer is $1x10^{8}$ pfu/ml (crude virus) or $1x10^{10}$ pfu/ml (concentrated virus).
4. **Efficient delivery:** the adenovirus can be transduced into actively dividing and non-dividing mammalian cells in culture or *in vivo*, and can obtain close to 100% expression in most cell types.

Transduction Protocols:

1. **Day 1**
   - Plate mammalian cells in complete medium under appropriate culture conditions.
   - Grow overnight.

2. **Day 2** *Cells should be ~ 50–75% confluent.*
   - Thaw the adenoviral stock at **room temperature** and dilute virus, if necessary, in PBS or complete medium, or make aliquots to save virus for subsequent use.
   - Add directly the appropriate amount of virus at a suitable MOI into the cell culture. Swirl the plate gently to evenly distribute the virus.
   - Incubate at 37°C overnight.

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3. **Day 3**

   Remove the medium containing virus and replace it with fresh, complete culture medium. This medium change is optional, depending on how toxic the virus is to the cells.

4. **2 or more days post-transduction**

   Harvest the cells, if needed, and assay for expression of your recombinant protein. The timing of this step will vary on cells, target protein, etc.

Safety Precaution:

Amsbio's adenovirus is replication incompetent; however, extra caution should always be taken with viral particles. Work with adenovirus in a Bio-safety II cabinet and wear gloves at all times when handling viral particles. Please refer to the CDC and NIH guidelines for more details regarding safety precautions.

Warranty and terms:

This product is for research use only. It is warranted to meet its quality criteria as described when used in accordance with its instructions. Amsbio disclaims any implied warranty of this product for particular applications. In no event shall Amsbio be liable for any incidental or consequential damages in connection with the products. Amsbio's sole remedy for breach of this warranty should be, at Amsbio's option, to replace the products.

The purchaser of this product is granted non-transferable right to use the purchased amount of the product in non-commercial use for the purchaser. No right to resell or re-produce this product is granted expressly or by implication.

References:

1. Update on Adenovirus and its Vectors. J. Gen. Virol. 81, 2573-2604, 2000. Russell, W. C., et al.
2. Supernatant Rescue Assay vs. Polymerase Chain Reaction for Detection of Wild Type Adenovirus-Contaminating Recombinant Adenovirus Stocks. J. Virol. Methods 56, 99-107, 1996, Dion, L. D, et al.
3. Construction and propagation of human adenovirus vectors. In Cell Biology: A Laboratory Handbook, Ed. Celis, J. E. (Academic Press, San Diego), pp. 500–512, 1998, Hitt, M.,, et al.

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FAQ for pre-made Adenovirus:

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

Pre-made Adenovirus is provided ready to use. Simply add it into the mammalian cell line of your choice. The amount of virus to add is dependent upon your cell types, or you 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 know the Adenovirus is working in my cells?

Adenovirus can transduce well the majority of mammalian cells --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 these types of cells, you may wish to try lentivirus. The best way to find out if your cells are susceptible to adenoviral transduction is to use a fluorescent control adenovirus, which will allow you to easily visualize transduction as fluorescence signal. Amsbio provides pre-made adenoviruses expressing a wide selection of fluorescent makers including GFP, RFP, YFP, and CFP, as well as fluorescent fusion markers.

3. How long will target expression last?

Typically, target expression can be detected after 12-24 hours. Depending on the cell type or cell cycle, the expression peaks at 2-3 days post-transduction and persists for as long as the viral genome is present, from 1 week to 6 months or longer.

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

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

5. What buffer is the virus provided in?

Amsbio adenoviruses are provided as 200 µl aliquots in two formats:

- Crude viral stock in DMEM medium with 10% serum, or

- Concentrated adenovirus re-suspended into PBS with 5% sucrose for *in vivo* use.

6. 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-2 level 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 an adenoviral transgene 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. We have not observed wild-type RCA contamination in small-scale amplifications. All our 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 cell death. To avoid the occurrence of RCA, viruses should be produced and amplified in low passage 293A cells.

7. How is viral titer measured?

The titer (IFU/ml) of 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 as measured by biological (plaque) assay, and is used as the reference titer for the non-fluorescent construct.

8. How are the adenovirus vectors constructed?

Amsbio uses its proprietary Eco cloning technology (vector built-in cloning competent cells) to directly insert a target into the E1/E3 deleted human adenovirus 5 genome.

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

Adenovirus is not integrated into the host cell genome; therefore, it is not suitable for long term expression and we do not include an antibiotic marker in adenovirus constructs. In some cases, we 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.

10. 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 transgene target will be expressed until the viral genome is diluted by cell division. Transgene 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.

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