

Adenoviral FAQs

Find out more about our adenoviral products, including vector systems, purification kits, and titration kits. Click on the expandable tabs below to view answers to the questions we are asked most frequently.



▼Questions about adenoviral vector systems

▼What is Adeno-X Adenoviral System 3?

Adeno-X Adenoviral System 3 from Takara Bio is the most advanced commercially available adenoviral gene delivery system. It is a single-vector-based system that allows you to clone a gene of interest (GOI) or custom-made expression cassette directly into the adenovirus backbone in a single step, using In-Fusion technology.

Table 1. Comparison of Adeno-X Adenoviral System 3 to the leading "easy" competitor			
	Adeno-X	Competitor	
Cloning time	2–3 days	8 days	
Cloning procedure	Simple	Complicated	
	• 30 min	Lots of hands-on time	
Cloning technology	In-Fusion HD	Homologous recombination in bacteria	
Subcloning into shuttle vector	Not required	Clone into a shuttle vector first	
Viral DNA yield	High	Low for the recombination strain	
E. coli strain	Stellar chemically competent cells (supplied)	2 strains required	
Cloning efficiency	9/10 clones correct	1/10 to 3/10 clones correct	
Screening	PCR-based	Miniprep followed by restriction digestion	
Inducible expression	Tightest control with Tet-On technology	Not available	
Monitor using fluorescent proteins	Red and green	Green only	
	Bright and consistent	Less bright	
Multiple fragment cloning	Single-step cloning	Clone in multiple steps	

▼What are the cloning/packaging capacities of Takara Bio's adenoviral vectors?

Vector cloning/packaging capacities vary, depending on the specific adenoviral vector backbone:







Table 2. Adeno-X systems & cloning capacities				
System		Cloning capacity (kb)		
Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)		4.6		
Adeno-X Adenoviral System 3 (CMV)		6.4		
Adeno-X Adenoviral System 3 (CMV, Red)		4.8		
Adeno-X Adenoviral System 3 (CMV, Green)		4.8		
Adeno-X Adenoviral System 3 (Universal)		8.0		
Adeno-X Adenoviral System 3 (Universal, Red)		6.4		
Adeno-X Adenoviral System 3 (Universal, Green)		6.4		

■What is the backbone of Takara Bio's adenoviral vectors?

Our adenoviral vectors carry deletions in the E1 and E3 regions of the adenovirus genome.

▼What type of adenovirus are Takara Bio's adenoviral vectors derived from?

Our adenoviral vectors are derived from human adenovirus type 5.

▼Do you have adenoviral vectors for tet-inducible gene expression?

Yes. We offer a third-generation Tet-On 3G inducible expression system in a single adenoviral vector format, the Adeno-X Adenoviral System 3 (Tet-On 3G Inducible).



- ▼Questions about adenoviral purification
- ▼What types of adenoviruses are compatible with Adeno-X purification kits?

Takara Bio has validated the Adeno-X purification kits for purification of Adeno-X viruses based on type 5 human adenovirus.

Can I purify adenovirus from cell supernatants or cells combined with supernatant using Adeno-X purification kits?

No. The Adeno-X Maxi/Mega purification kits only purify virus from cell pellets, which accumulate most of the amplified virus, provided that the cells are harvested prior to complete cell lysis (Figure 1). This protocol greatly simplifies adenoviral purification.







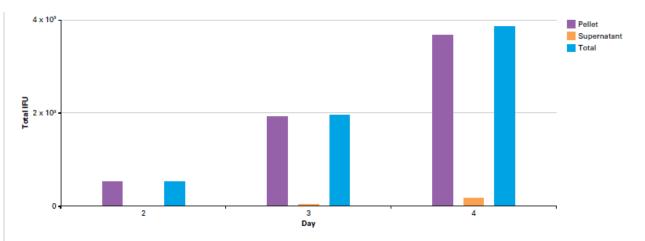


Figure 1. Time course of adenoviral yields from cell pellets and supernatants. HEK 293 cells were transduced with an Adeno-X construct expressing DsRed-Express and incubated for 2–4 days. On Days 2–4, cells were harvested, lysed, and centrifuged—and each pellet (containing the cell's contents) and supernatant (containing the medium the cells were growing in) was used to infect fresh HEK 293 cells to measure titer. Most of the virus was found in the cell pellets, especially by Day 4, with only 2% in the supernatant.

Can I use adenovirus purified with Adeno-X purification kits for in vivo studies?

Yes. Adenovirus purified with Adeno-X purification kits can be used for *in vivo* experiments after desalting by column chromatography or dialysis. We recommend using the Adeno-X Mega Purification Kit (Cat. # 631032) to purify adenovirus for *in vivo* experiments.



Questions about adenoviral titration

What types of adenoviruses are compatible with the Adeno-X Rapid Titer Kit?

The Adeno-X Rapid Titer Kit is compatible with human adenoviruses (types 1, 2, 5, and 6). Please note that we have validated the Adeno-X Rapid Titer Kit for titration of Adeno-X viruses, based on type 5 human adenovirus.

▼What is MOI?

MOI, or multiplicity of infection, is the number of infectious viral units per cell at the time of infection.

▼What is IFU?

IFU, or infectious units, determines the virus' ability to infect cells and express protein. You can determine the infectious viral titer (IFU/mI) by measuring the expression of a reporter or a specific protein by flow cytometry, immunostaining, etc.

▼What is PFU?

PFU, or plaque forming units, measures the ability of the virus to infect permissive cells and replicate, forming plaques (holes that represent lysed cells) in the cell monolayer. The titer in PFU/ml is determined by a plaque assay.





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