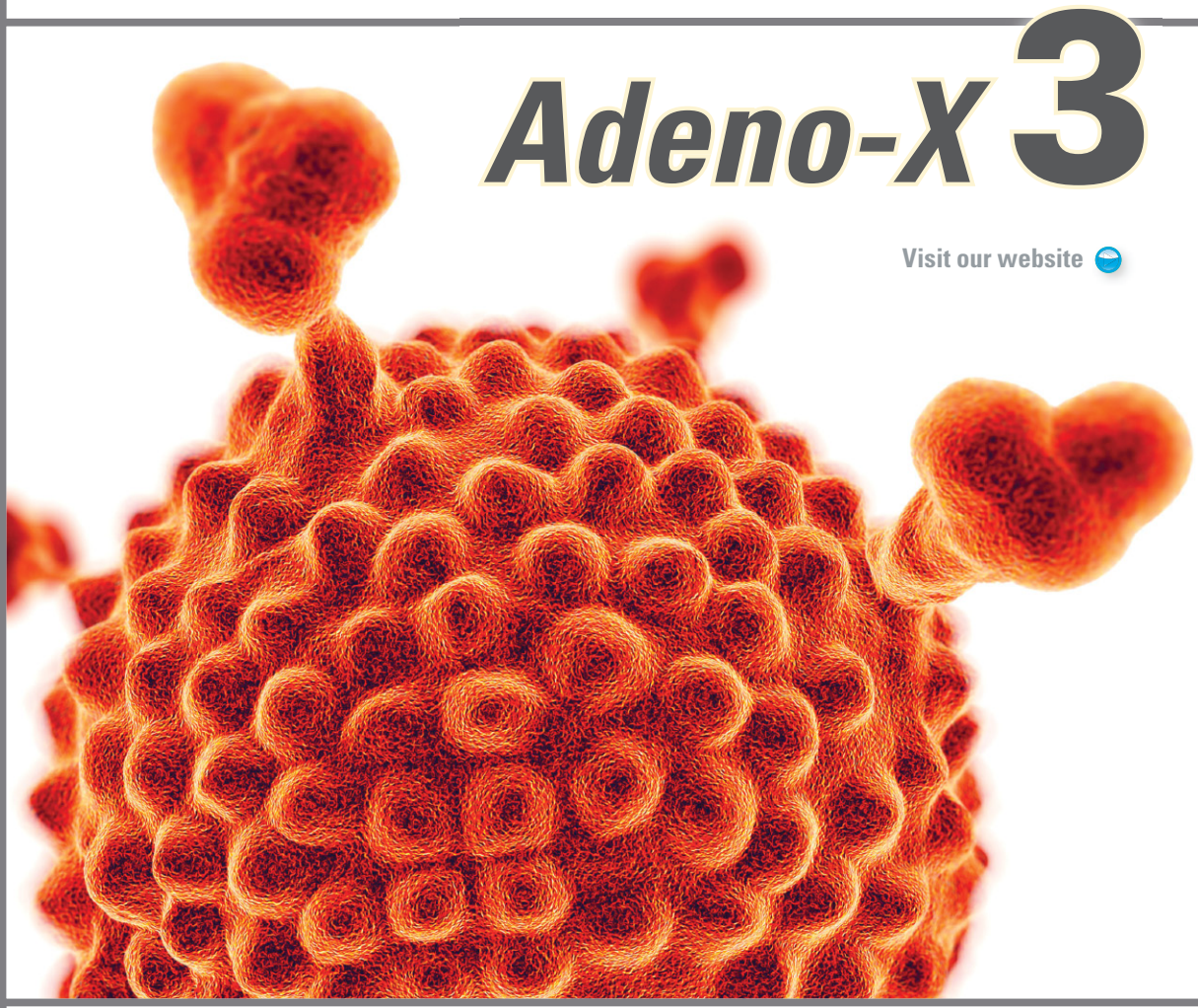
Adenoviral Expression Systems

Lentivirus is not the only choice for gene delivery



Clontech

Why choose adenoviral gene delivery?

Table I: Adenoviral vs. Lentiviral Gene Delivery		
	Lentivirus	**Adenovirus**
Infects many different human cell types	Yes	Yes
Infects both dividing and non-dividing cells	Yes	Yes
Non-integrating virus	No	Yes
High level of protein expression (up to 10–20% total protein)	No	Yes
Ability to accommodate long inserts (up to 8 kb)	No	Yes
Easy to scale-up/amplify	No	Yes
Easy to get titers >10^9 IFU/ml	No	Yes
Easy to get a multiplicity of infection >25 copies per cell	No	Yes

Transduce primary cells and transformed cell lines: Infection by adenovirus is not cell cycle-dependent, so you can deliver your gene to primary cells as well as transformed cell lines.

Very high titers: Recombinant adenoviruses such as Adeno-X™ are lytic only in packaging cells that provide the essential E1 protein *in trans* (such as HEK 293 cells). This lytic mechanism of amplification means that virus particles produced by one cell can reinfect adjacent packaging cells to produce a cascade of virus production and ultimately far higher titers (>10^9 IFU/ml) than can be achieved with any recombinant lentivirus system.

Easy to reamplify: Simply add your virus stock to a dish of Adeno-X 293 cells, wait a few days, and harvest again. No additional transfection or optimization is needed.

Very high expression levels are possible: Overexpress protein delivered by adenovirus prior to purification from mammalian cells. Following infection, transduced cells can receive multiple copies of the recombinant genome, so expression can be 10–20% of total protein, far higher than from a lentiviral system.

What is different about Adeno-X Adenoviral System 3?

Faster and easier: Until now the main drawback of commercially supplied adenoviral vector systems has been the need to use complex cloning procedures to overcome the difficulties with cloning into large (~34 kb) plasmids. At Clontech, our Adeno-X virologists thought *"Wouldn't it be great if you could clone directly into the adenoviral plasmid just like any plasmid?"* They then harnessed the power of In-Fusion® HD cloning technology to make this happen (Figure 1).

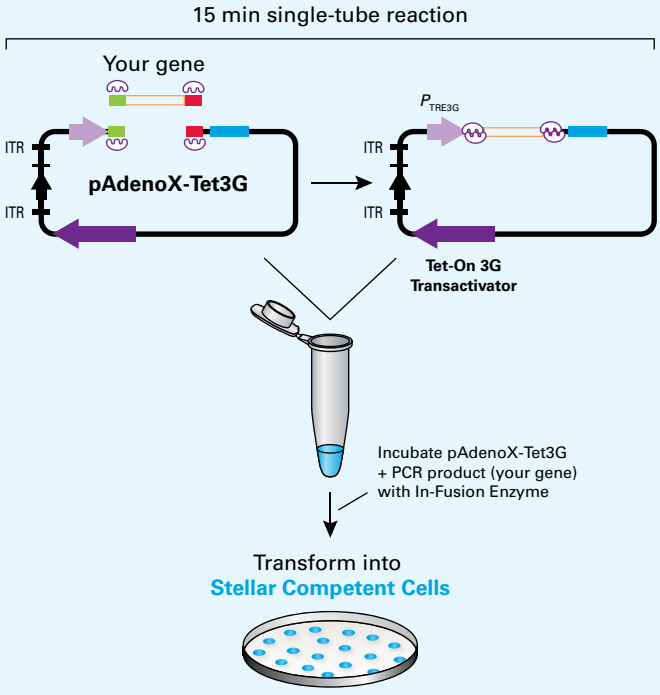


Figure 1. Clone into adenovirus just like any other plasmid!

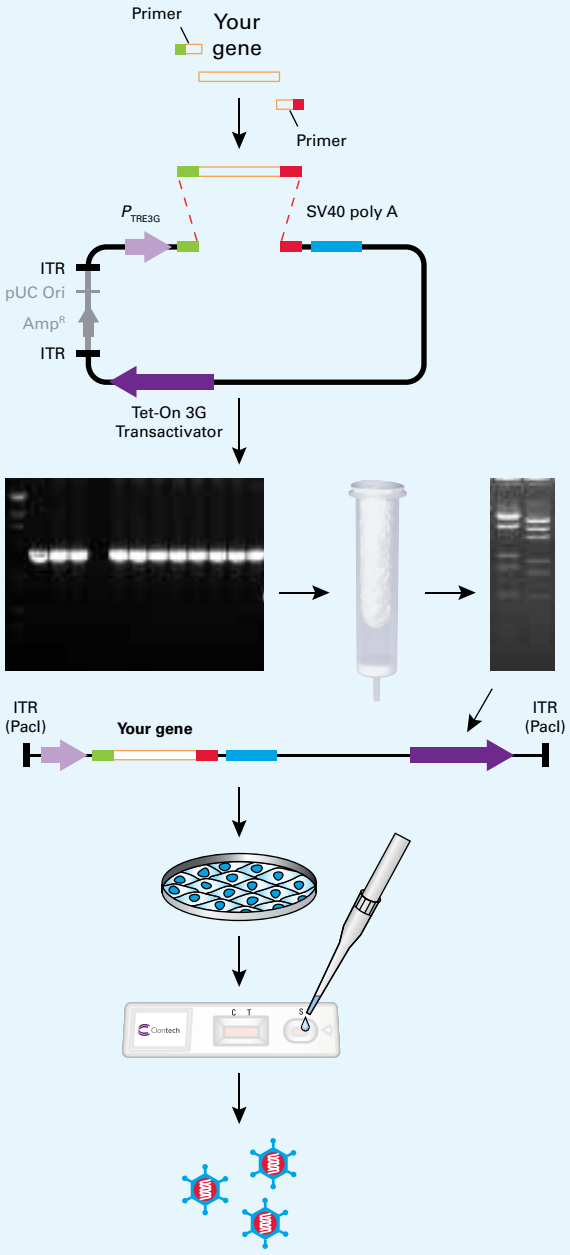
Table II: Adeno-X Adenoviral System 3 Features	
Feature	**Description**
No shuttle vector required	Clone directly into the adenoviral vector using In-Fusion HD
Easy to use	As easy as any plasmid cloning system
Fast	2–3 day cloning (other "easy" systems take at least 8 days)
Super high-efficiency cloning	Hundreds of colonies, 9/10 clones are correct
Highly flexible formats	Use an existing expression cassette or create one without additional subcloning
Best technologies offered	Tet-On® 3G Inducible Expression, fluorescent reporters, multiple fragment cloning

Multiple formats are available

Adeno-X Adenoviral System 3 is available in seven formats (see Ordering Information), including...

- The most advanced tetracycline inducible expression system
- Constitutive expression systems
- With or without fluorescent reporters
- Universal systems that allow you to clone and express any entire expression cassette of your choice

There is no simpler adenoviral expression system



1. **Amplify your gene with 15 bp of homology to pAdenoX**
2. **Clone using In-Fusion technology and Stellar Competent Cells**
3. **Perform colony PCR to verify correct clone(s)**
4. **Purify the pAdenoX vector containing your gene insert using NucleoBond Xtra**
5. **Digest with XhoI/NheI to verify integrity**
6. **Digest with PacI (to linearize)**
7. **Transfect Adeno-X 293 cells**
8. **Confirm the presence of adenovirus with Adeno-X GoStix**
9. **Harvest recombinant adenovirus**

Figure 2. Constructing recombinant adenovirus using the Adeno-X Adenoviral System 3. DNA sequences can be rapidly transferred as PCR products to any pAdenoX vector using the In-Fusion cloning method. In this example, your gene of interest is amplified with 15 bp extensions that are homologous to the ends of the linearized adenoviral vector. The PCR product is then purified and mixed with the linearized adenoviral vector of choice in the In-Fusion reaction. Following the reaction, a portion of the mixture is transformed into *E. coli* (Stellar™ Competent Cells) and screened. Once a PCR-positive clone is identified, the recombinant pAdenoX vector is amplified, purified, and subsequently linearized with the restriction enzyme PacI, then transfected into Adeno-X 293 cells for viral rescue and amplification. Adeno-X GoStix™ can be used to determine the status of adenovirus rescue.

Tetracycline inducible expression

When you clone your gene into pAdenoX-Tet3G, you are creating a system with the tightest and most sensitive control of gene expression. Tightly-controlled, doxycycline-induced expression is as easy as constitutive expression since the Tet-On 3G transactivator gene and the P_{TRE3G}-controlled gene of interest are present on the same adenoviral vector (Figure 3). Up to 3,000-fold induction can be achieved using this system (Figure 4).

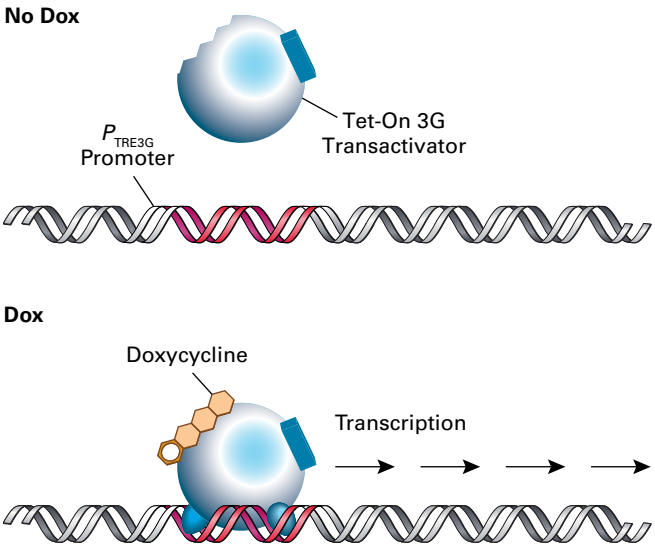


Figure 3. The Tet-On 3G Systems allow inducible gene expression only in the presence of doxycycline.

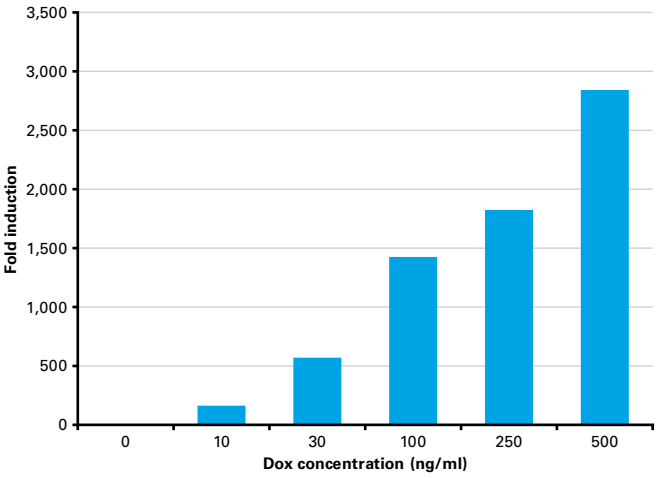


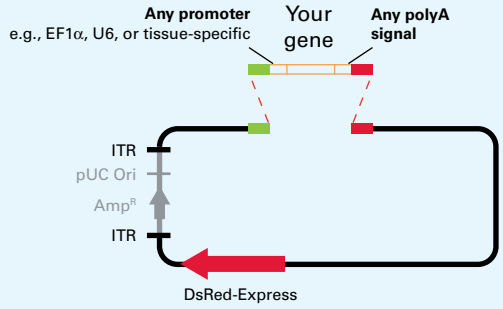
Figure 4. The Adeno-X Tet-On 3G Systems generate very high-fold induction, with up to a 3,000-fold difference between induced and uninduced states. Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were infected with Adeno-X Tet-On 3G Luciferase virus. Cultures were harvested and assayed for luciferase activity.

Clone any expression cassette into the Universal vectors

Universal vectors lack a promoter and polyA signal and can be used to drive expression of constructs from the promoter of your choice. Simply amplify an entire expression cassette (from promoter to polyA) from a pre-existing construct and clone using In-Fusion HD (Figure 5, Panel A). Alternatively, you may wish to transfer your shRNA or miRNA expression cassette from a pre-existing plasmid to one of the universal pAdenoX vectors in order to create a high-efficiency RNAi delivery system. You can create a unique expression cassette using multiple fragment cloning (Figure 5, Panel B), with only a small loss in cloning efficiency (Figure 5, Panel C).

A

Complete expression cassette cloned into a Universal system



B

Multiple fragment cloning in a Universal system

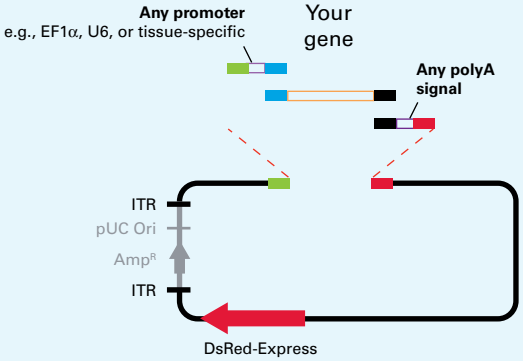


Figure 5. The Universal Adeno-X Expression Systems contain vectors that lack a promoter and polyA signal in the cloning site. You can either clone an expression cassette from a preexisting construct into the vector **(Panel A)** or create a new one using multiple fragment In-Fusion HD cloning **(Panel B).**

C

Table III: Multiple Fragment Cloning Efficiency with Adeno-X Adenoviral System 3

	Number of colonies (1/10th plated)	Percentage correct (%)
pAdenoX + 1 insert	200–300	90
pAdenoX + 2 inserts	200–300	60
pAdenoX + 3 inserts	25–40	25

Adenovirus Transduction Tools

Titration Kits

- The **Adeno-X Rapid Titer Kit** allows functional, hexon-based titration
- The **Adeno-X qPCR Titration Kit** allows ultra-fast, qPCR-based titration

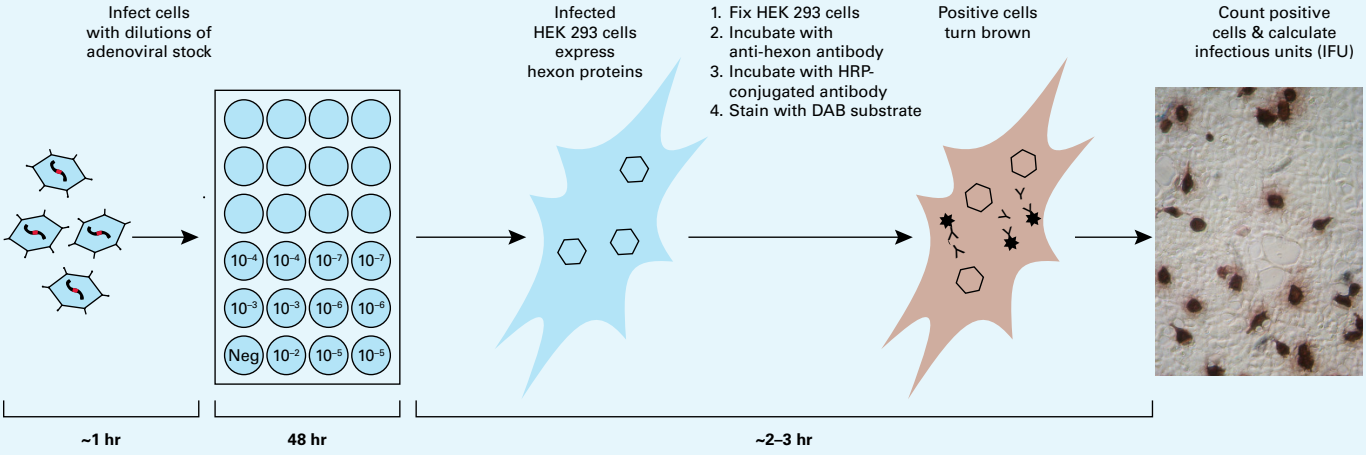


Figure 6. The Adeno-X Rapid Titer Kit provides a quick and simple antibody-based assay that can be used to measure functional adenovirus titers. The kit is designed around a hexon-specific antibody, which is used to label infected cells that can then be easily counted.

Adeno-X GoStix: rapid and instant adenovirus detection during early amplification

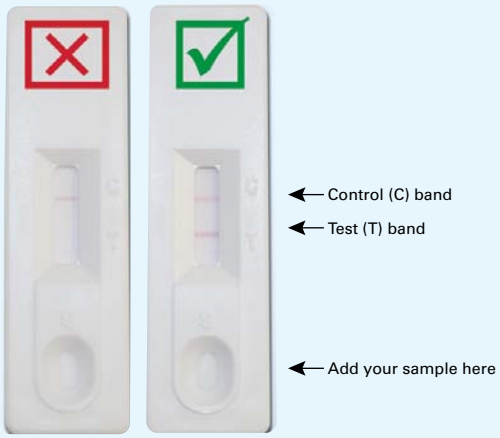


Figure 7. Sensitive and unambiguous detection of adenovirus with Adeno-X GoStix. Following the addition of cell culture supernatant, the test band (T) shows positive only if there is enough virus in your sample, while the control band (C) is always detected.

Purification Kits

- Adeno-X Maxi Purification Kit: purify up to 10^{12} adenoviral particles from 5 x 15 cm plates
- Adeno-X Mega Purification Kit: purify up to 10^{13} adenoviral particles from 25 x 15 cm plates

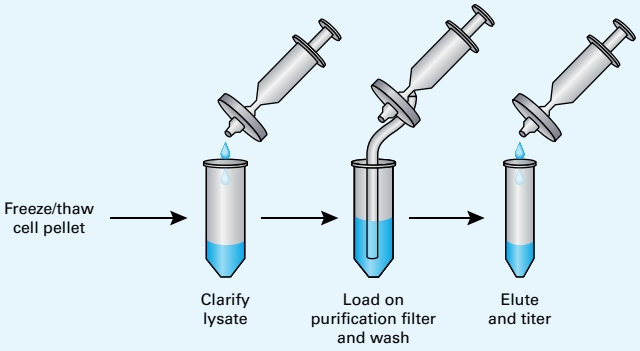
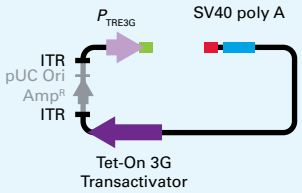
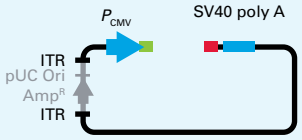
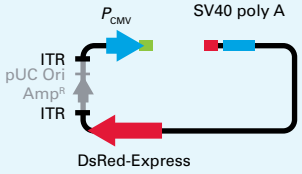
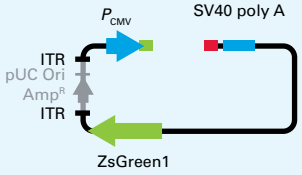
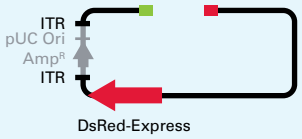
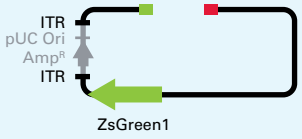


Figure 8. Our adenovirus purification protocol contains no ultra-centrifugation steps. Instead, adenovirus is purified chromatographically using a unique membrane that selectively binds adenoviral particles.

Adeno-X 293 Cells

Compared to most laboratory strains of HEK 293, Adeno-X 293 exhibits a slower growth rate and are more strongly adherent, features that lead to more efficient rescue and amplification during adenovirus production.

Adeno-X Adenoviral System 3 Formats & Ordering Information

Product	Description	Vector Map	Cat. No.
Adeno-X Adenoviral System 3 (Tet-On 3G Inducible)	• Tightly-controlled, doxycycline-inducible expression system		631180
Adeno-X Adenoviral System 3 (CMV)	• Constitutive expression from a CMV promoter		632269
Adeno-X Adenoviral System 3 (CMV, Red)	• Constitutive expression from a CMV promoter • Red fluorescent protein to easily monitor transfection and transduction		632268
Adeno-X Adenoviral System 3 (CMV, Green)	• Constitutive expression from a CMV promoter • Green fluorescent protein to easily monitor transfection and transduction		632267
Adeno-X Adenoviral System 3 (Universal)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA		632266
Adeno-X Adenoviral System 3 (Universal, Red)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA • Red fluorescent protein to easily monitor transfection and transduction		632265
Adeno-X Adenoviral System 3 (Universal, Green)	• Use any promoter and any polyA sequence • Ideal for tissue-specific expression or expression of shRNA or miRNA • Green fluorescent protein to easily monitor transfection and transduction		632264

Ordering Information

Product	Size	Cat. No.
HEK 293 Cells for Adenoviral Packaging		
Adeno-X 293 Cell Line	Each	632271
Adenovirus Purification Kits		
Adeno-X Maxi Purification Kit	2 preps 6 preps	631532 631533
Adenovirus Titration Kits		
Adeno-X Rapid Titer Kit	120 titrations	632250
Adeno-X qPCR Titration Kit	Each	632252
Adeno-X GoStix	20 Tests	632270

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