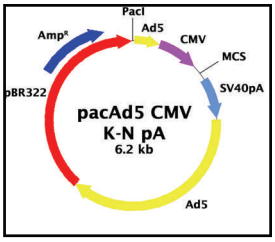
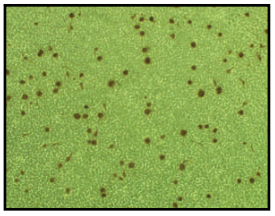
Adenoviral Expression

Choose your Viral Vector for Gene Delivery				
	Adenovirus	Adeno-Associated Virus (AAV)	Lentivirus (HIV-1, FIV, SIV)	Retrovirus (MMLV)
Gene Expression	**Transient**	Transient or Stable	Transient or Stable	Stable
Infect Dividing Cells	**Yes**	Yes	Yes	Yes
Infect Non-dividing Cells	**Yes**	Yes	Yes	No
Integration into Target Cell Genome	**No**	Yes	Yes	Yes
Immune Response in Target Cells	**High**	Very Low	Low	Moderate
Relative Viral Titer	**XXXX**	XXX	XXX	XX
Relative Transduction Efficiency	**XXXX**	XXX	XXX	XX

Adenoviral Expression Procedure

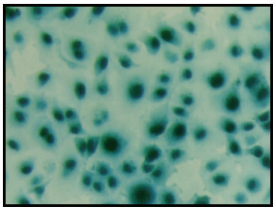


Package Virus with Gene of Interest
(p. 2)
-OR-
Obtain Premade Virus containing Gene of Interest
(p. 3)



Measure Viral Titer
(p. 4)

Purify your Adenovirus
(p. 5)



Infect Target Cells
(p. 6)

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Generate high-titer adenovirus in a fraction of the time with substantially reduced replication-competent virus

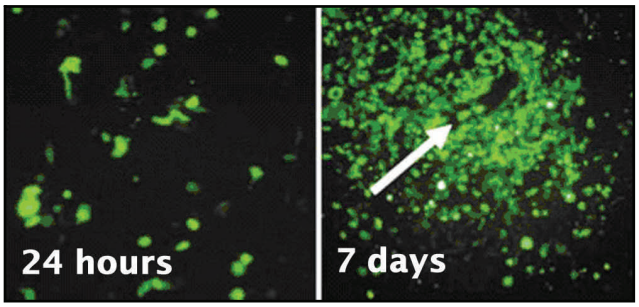
RAPAd® Adenoviral Expression Systems

While recombinant adenovirus provides a robust method of gene delivery, generating the recombinant adenovirus itself can be slow and difficult. Traditional homologous recombination takes months; newer commercial methods take less time, but can produce significant amounts of replication-competent adenovirus (RCA). Levels of RCA can increase to dangerous levels with serial amplification.

The RAPAd® Adenoviral Expression Systems produce recombinant adenovirus containing your gene of interest in a very short 2-3 week period, compared to 8-18 weeks required by other systems.

The backbone vector in the RAPAd® systems is engineered to produce <300 wild type plaques per 10^9 viral particles, substantially lower than the 10^4 to 10^6 WT plaques per 10^9 particles produced by most other methods.

RAPAd® Adenoviral Expression Systems are available in 5 formats: Universal (promoterless), RSV promoter, CMV promoter, CMV/GFP bicistronic, and miRNA. The Universal format allows you to clone in your own promoter along with your gene of interest.



Generation of recombinant adenovirus using the RAPAd® Adenoviral Expression System.

Standard Homologous Recombination	pAdEasy™ Expression System	**RAPAd® Expression System**
Cotransfect 293 cells with Shuttle Vector and Ad Backbone Vector	Linearize Shuttle Vector using PmeI	Linearize Shuttle Vector and Ad Backbone Vector using PacI
Multiple Plaque Isolations	Cotransform BJ5183 cells with linearized Shuttle Vector and pAdEasy Vector	Cotransfect 293 cells
Virus Amplification	Recombinant selection by restriction enzyme analysis	Viral Stock
Viral Stock	Linearize recombinant plasmid using PacI	
	Transfect 293 cells	
	Viral Stock	
12-18 weeks	**8-9 weeks**	**2-3 weeks**

Comparison of available methods for production of recombinant adenovirus.

When packaging your adenovirus, consider our **293AD Adenoviral Cell Line**. This cell line is derived from the parental HEK 293 cell line, but has been specifically selected for use in adenoviral expression. You'll see firmer attachment to culture plates, flattened morphology, and a much larger surface area for higher viral yields and superior transfection.

High-titer adenoviruses already containing a gene of interest; ideal if you're studying multiple genes or mutation states

Premade Recombinant Adenoviruses

If you don't have time to make your own adenovirus constructs, rely on our premade adenoviruses that contain your gene of interest. All premade adenoviruses are provided at a high concentration of 10^{11} viral particles/mL.

Currently we offer over 100 recombinant adenoviruses containing genes involved in many different pathways, including the following (a complete list may be found at the back of this brochure):

Controls and Reporters

- ß-Galactosidase
- Cre
- Empty (Null) control
- GFP
- Luciferase
- SEAP

Cytoskeleton / Small GTPase (wt and mutants)

- Cdc42
- PAK1
- Rac
- Ras
- RhoA
- SDF-1alpha

MAP Kinase (wt and mutants)

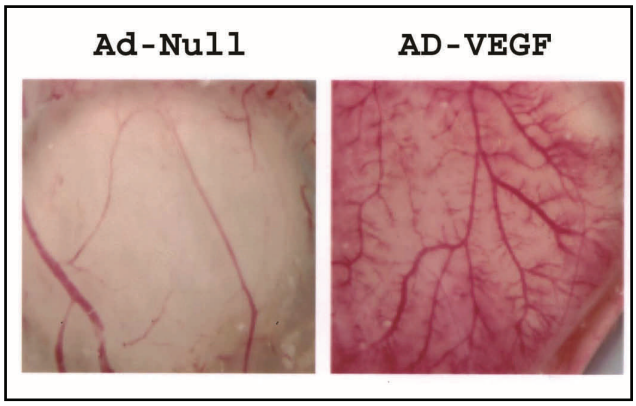
- ERK2, ERK5
- IFN-gamma
- IL-2
- JNK1
- MAPKAPK2
- MEK1, MEK5
- MEKK1, MEKK3
- MKK3, MKK4, MKK6, MKK7
- p38 isoforms
- PRAK
- Raf1
- SOK

NFkB Signaling

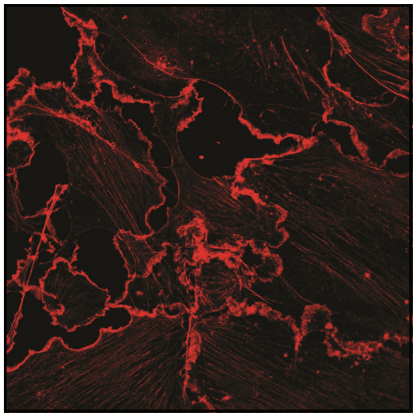
- IkB-alpha
- IKK-ß
- NOD2
- Rel B

Cell Cycle

- DCC
- MyoD
- Myogenin
- p53



Blood Vessel Formation After 3 Days in Presence of Ad-Null (#ADV-001) or Ad-VEGF (#ADV-101) in 10-day old Chick Chorioallanoic Membrane.



Actin Cytoskeleton Staining of Cos-7 Cells Infected with Purified Ad-Ras V12 (#ADV-146) at 50 MOI.

Additional Targets

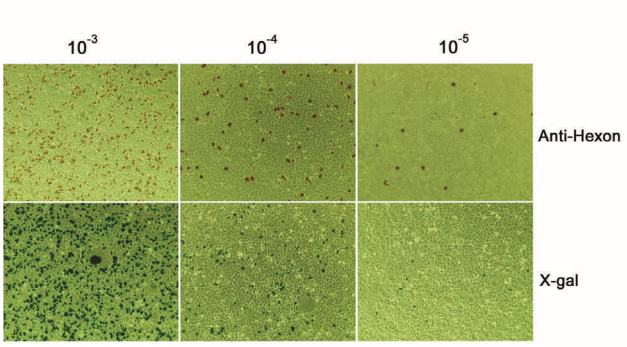
- Akt1
- CA9
- CEA
- CSK
- Fyn
- HIF-1alpha
- NY-ESO-1
- PKC isoforms
- Src
- VEGF

Faster and more accurate than plaque assays; measure functional or physical viral titer

QuickTiter™ Adenovirus Quantitation Kits

Once you've made your adenovirus, you will want to measure the titer of your virus prior to infecting your target cells. Knowing that this has been traditionally done with a tedious plaque forming unit (PFU) assay that takes nearly 2 weeks, you might again be tempted to skip this step.

Our QuickTiter™ Adenovirus Quantitation Kits make the titer process extremely simple in a small fraction of the time. Depending on your needs, you can obtain a physical or a functional titer of your adenovirus.



Immunostaining of Ad-ß-Gal in 293AD cells using the QuickTiter™ Adenovirus Titer Immunoassay Kit.

Functional Titer

QuickTiter™ Adenovirus Functional Titer Kits make quantifying your virus much easier and faster. In just 2.5 days, you can get a more accurate titer of your adenovirus in infectious units/mL with these antibody-based assays. See your results by either ICC staining or ELISA.

Physical Titer

If 2.5 days is still too long, and you don't need an infectious titer of your adenovirus, the QuickTiter™ Adenovirus Quantitation Kit is made for you. This kit measures the viral nucleic acid content. In just under 1 hour, you can measure the physical titer of your virus on a fluorescence plate reader.

Select your QuickTiter™ Quantitation Kit

	QuickTiter™ Adenovirus Titer Immunoassay Kit (#VPK-109)	QuickTiter™ Adenovirus Titer ELISA Kit (#VPK-110)	QuickTiter™ Adenovirus Quantitation Kit (#VPK-106)
Functional or Physical Titer	Functional (Infectious units)	Functional (Infectious units)	Physical (Viral particles)
Assay Time	2.5 days	2.5 days	45-60 minutes
Assay Principle	Antibody-based	Antibody-based	Measures nucleic acid content
Detection Method	Immunocytochemical staining	Colorimetric (ELISA) plate reader	Fluorescence plate reader
Kit Size	100 assays	2 x 96 assays	20 assays

Fast, high-yield alternative to cesium chloride preparations; never ultracentrifuge your adenovirus supernatant again!

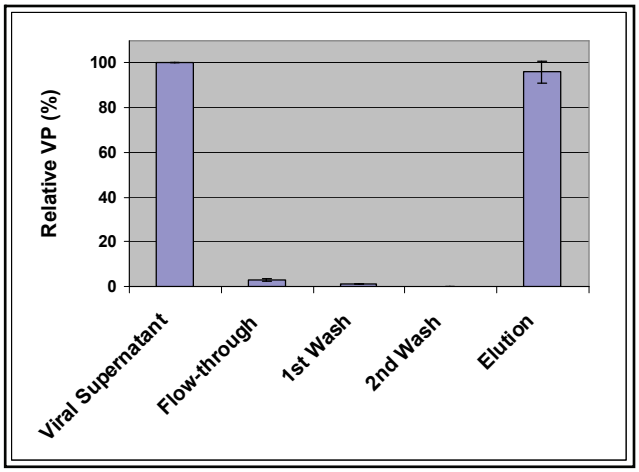
ViraBind™ Adenovirus Purification Kits

Purification of your adenovirus is important in obtaining good gene expression results. However, the thought of tedious, time-consuming ultracentrifugation might tempt you to skip this crucial step.

ViraBind™ Adenovirus Purification Kits make purification fast, easy and highly efficient. These kits deliver high-yield adenovirus in about 30 minutes without the need for an ultracentrifuge. Obtain highly pure adenovirus with >90% recovery rate.

ViraBind™ Adenovirus Purification Kits are available in two sizes:

- Our standard purification kit uses a proprietary syringe filter with an extremely high binding capacity, allowing maximum recovery with minimal sample loss
- Our Miniprep kit uses a special spin column to purify smaller volumes of adenovirus quickly and efficiently



Purification of Recombinant Ad-β-Gal Using ViraBind™ Adenovirus Purification Kit (#VPK-100).

Tip: After purifying your virus, it is a good practice to measure your viral titer again, regardless of the purification method used.

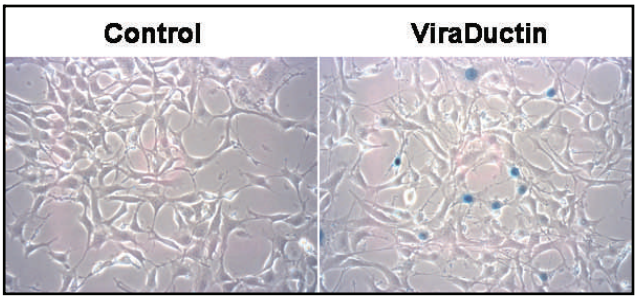
Select your ViraBind™ Purification Kit		
	ViraBind™ Adenovirus Miniprep Kit (#VPK-099)	**ViraBind™ Adenovirus Purification Kit (#VPK-100)**
Purification Method	Spin column	Syringe filter
Purification Time	30 minutes	30 minutes
Capacity / Prep (Viral Particles)	1 x 10^{11} VP	2.5 x 10^{12} VP
Capacity / Prep (Supernatant Qty)	One T75 flask or one 10cm dish	Four T75 flasks
Kit Size	10 Preps	10 Preps

Easily increase your infection efficiency in cells lacking the coxsackievirus-adenovirus receptor (CAR)

ViraDuctin™ Adenovirus Transduction Reagent

After you've determined the titer of your purified adenovirus, it is time to infect your target cells. Some cells express the coxsackievirus-adenovirus receptor (CAR), which is the primary means of adenoviral infection. In many cells, however, expression of CAR is low or non-existent. This makes infection of your target cell more difficult.

The ViraDuctin™ Adenovirus Transduction Reagent overcomes this obstacle to infection. This reagent specifically increases the efficiency of adenoviral transduction, without regard to the expression level of CAR on the surface of the target cell. The reagent requires just a short incubation step prior to target cell infection, and can result in up to 12-fold higher uptake of your adenovirus.



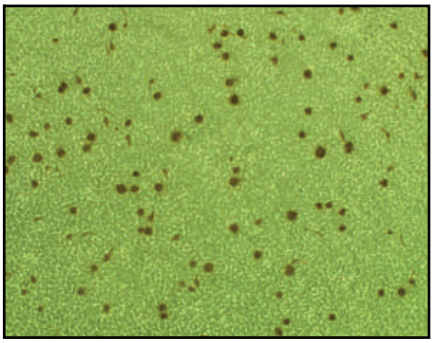
Enhanced Transduction of Ad-ß-Gal using ViraDuctin™ Adenovirus Transduction Reagent on NIH3T3 Cells.

Detect replication-competent adenovirus in substantially less time

Replication-Competent Adenovirus Assay

In some adenoviral expression experiments, it may be critical to measure the presence of replication-competent adenovirus (RCA) in your sample. Traditionally this is done via the 10-14 day plaque-forming unit (PFU) assay mentioned previously.

Our Rapid RCA Assay kit streamlines this assay to a 2-day incubation with detection by immunostaining. The assay principle is similar to that of our QuickTiter™ Adenovirus Immunoassay Titer Kit (see previous page).



Immunostaining of wild type Ad5 using the Rapid RCA Assay Kit.

Ordering Information and Published Citations

Premade Recombinant Adenoviruses

All viruses provided as 50 µL. CA = Constitutively Active; DN = Dominant Negative; FSM = Frame Shift Mutation; TSM = Temp. Sensitive Mutant

Target Name	Catalog #
Null (no gene)	ADV-001
ß-Galactosidase	ADV-002
CA9	ADV-602
Cdc42	ADV-152
Cdc42 L61 (CA)	ADV-154
Cdc42 N17 (DN)	ADV-153
CEA	ADV-604
Cre	ADV-005
CSK	ADV-405
CSK (DN)	ADV-406
DCC	ADV-504
ERK2	ADV-112
ERK2 (DN)	ADV-113
ERK5	ADV-116
ERK5 (DN)	ADV-117
Fyn	ADV-403
Fyn (DN)	ADV-404
GFP	ADV-004
HIF-1α	ADV-100
IFN-γ	ADV-103
IκB-α	ADV-301
IκB-α S32A (DN)	ADV-302
IKK-ß	ADV-305
IKK-ß (DN)	ADV-303
IL-2	ADV-102
JNK1	ADV-114
JNK1 (DN)	ADV-115
Luciferase (Firefly)	ADV-008
MAPKAPK2	ADV-137
MAPKAPK2 (DN)	ADV-138
MAPKAPK2 (CA)	ADV-139
MEK1 (DN)	ADV-118
MEK1 (CA)	ADV-119
MEK5	ADV-129
MEK5 (DN)	ADV-130
MEK5 (CA)	ADV-131
MEKK1	ADV-135
MEKK1 (DN)	ADV-136
MEKK3	ADV-162

Target Name	Catalog #
MKK3	ADV-120
MKK3 (DN)	ADV-121
MKK3 (CA)	ADV-122
MKK4 (DN)	ADV-160
MKK4 (CA)	ADV-161
MKK6	ADV-123
MKK6 (DN)	ADV-124
MKK6 (CA)	ADV-125
MKK7	ADV-126
MKK7 (DN)	ADV-127
MKK7 (CA)	ADV-128
MyoD	ADV-508
Myogenin	ADV-509
myr-Rac1	ADV-163
NOD	ADV-308
NOD (FSM)	ADV-309
NY-ESO-1	ADV-601
p38α	ADV-104
p38α (DN)	ADV-105
p38ß	ADV-106
p38ß (DN)	ADV-107
p38γ	ADV-108
p38γ (DN)	ADV-109
p38δ	ADV-111
p53	ADV-501
p53 (TSM)	ADV-502
p68 RNA Helicase	ADV-505
PAK1	ADV-202
PAK1 H83L, H86L	ADV-203
PAK1 H83L, H86L, K299R	ADV-205
PAK1 K299R	ADV-207
PAK1 Kinase Domain	ADV-209
PAK1 L107E, T423E	ADV-206
PAK1 Regulatory Domain	ADV-208
PAK1 T423E	ADV-204
PKC-α (DN)	ADV-410

Recent Product Citations

1. Ackerman, W. et al (2008). Nuclear Factor-kappa B regulates inducible prostaglandin E synthase expression in human amnion mesenchymal cells. *Biol. Reprod.* **78**:68-76. (ADV-002, ADV-302)
2. Jones, S.W. et al (2009). Mitogen-activated protein kinase-activated protein kinase (MK2) modulates key biological pathways associated with OA disease pathology. *Osteoarthritis and Cartilage* **17**:124-131. (ADV-004, ADV-105)
3. Stoletov, K. et al (2010). Visualizing extravasation dynamics of metatstatic tumor cells. *J. Cell Sci.* **123**:2332-2341. (ADV-101)
4. Nigro, P. et al (2010). PKC-zeta decreases eNOS protein stability via inhibitory phosphorylation of ERK5. *Blood* **116**:1971-1979. (ADV-112)
5. Monick, M. et al (2008). Constitutive ERK MAPK activity regulates macrophage ATP production and mitochondrial integrity. *J. Immunol.* **180**:7485-7496. (ADV-112, ADV-113, ADV-118, ADV-119)
6. Yoon, C-H. et al (2009). Activation of p38 mitogen-activated protein kinase is required for death receptor-independent caspase-8 activation and cell death in response to sphingosine. *Mol. Cancer Res.* **7 (3):**361-370. (ADV-119)
7. Tan, S.H. et al (2009). Regulation of cell proliferation and migration by TAK1 via transcriptional control of von Hippel-Lindau tumor suppressor. *J. Biol. Chem.* **284**:18047-18058. (ADV-128)
8. Black, S.A. et al (2008). TGFß1 stimulates connective tissue growth factor (CCN2/CTGF) expression in human gingival fibroblasts through a RhoA-independent, Rac1/Cdc42-dependent mechanism: statins with forskolin block TGFß1-induced CCN2/CTGF expression. *J. Biol. Chem.* **283**:10835-10847. (ADV-145, ADV-150, ADV-153, ADV-156)
9. Thomas, M.A. et al (2009). E4orf1 limits the oncolytic potential of the E1B-55K-deleted adenovirus. *J. Virol.* **83**:2406-2416. (ADV-150)
10. Cheng, Z-J. et al (2010). Co-regulation of caveolar and Cdc42-dependent fluid phase endocytosis by phosphocaveolin-1. *J. Biol. Chem.* **285**:15119-15125. (ADV-153)
11. Fang, W.B. et al (2008). Overexpression of EPHA2 receptor destabilizes adherens junctions via a RhoA-dependent mechanism. *J. Cell Sci.* **121**:358-368. (ADV-157)
12. Taniguchi, C. et al (2007). The p85a regulatory subunit of phosphoinositide 3-kinase potentiates c-Jun N-terminal kinase-mediated insulin resistance. *Mol. Cell Biol.* **27**:2830-2840. (ADV-161)
13. Richardson, W.M. et al (2010). Nucleotide-binding oligomerization domain-2 inhibits toll-like receptor-4 signaling in the intestinal epithelium. *Gastroenterology* 10.1053/j.gastro.2010.05.038. (ADV-308, ADV-309)
14. Koh, W. et al (2009). Formation of endothelial lumens requires a PKC -, Src-, PAK-, and Raf-kinase dependent signaling cascade downstream of Cdc42 activation. *J. Cell Sci.* **122**:1812-1822. (ADV-401, ADV-405, ADV-406)

Target Name	Catalog #
PKC-θ (DN)	ADV-411
PKC-ζ (DN)	ADV-412
PRAK	ADV-141
Rac1	ADV-149
Rac1 L61 (CA)	ADV-150
Rac1 N17 (DN)	ADV-151
Raf1	ADV-132
Raf1 (DN)	ADV-133
Raf1 (CA)	ADV-134
Ras N17 (DN)	ADV-145
Ras V12 (CA)	ADV-146
Ras V12C40	ADV-148
Ras V12S35	ADV-147

Target Name	Catalog #
RelB	ADV-304
RhoA L63 (CA)	ADV-157
RhoA N19 (DN)	ADV-156
SDF-1α	ADV-210
SEAP	ADV-003
shAkt1	ADV-417
shAkt2	ADV-418
SOK	ADV-142
SOK (DN)	ADV-143
SOK (CA)	ADV-144
Src	ADV-401
Tac-Rac1	ADV-164
VEGF	ADV-101

Ordering Information and Published Citations

Adenoviral Expression Systems

Product Name	Promoter	Size / Qty	Catalog Number
RAPAd® Universal Adenoviral Expression System	None	1 Kit	VPK-250
RAPAd® RSV Adenoviral Expression System	RSV	1 Kit	VPK-251
RAPAd® CMV Adenoviral Expression System	CMV	1 Kit	VPK-252
RAPAd® miRNA Adenoviral Expression System	EF-1	1 Kit	VPK-253
RAPAd® Bicistronic Adenoviral Expression System (GFP)	CMV	1 Kit	VPK-254
293AD Cells	N/A	10^6 Cells	AD-100

Recent Product Citation

Kothari, H. et al (2007). Cystine 186-cystine 209 disulfide bond is not essential for the procoagulant activity of tissue factor or for its de-encryption. *Blood* **115**:4273-4283. (AD-100)

Adenovirus Quantitation & Titer Kits

Product Name	Detection	Size / Qty	Catalog Number
QuickTiter™ Adenovirus Immunoassay Titer Kit	Immunostaining	100 Assays	VPK-109
QuickTiter™ Adenovirus Immunoassay ELISA Kit	Colorimetric	2 x 96 Assays	VPK-110
QuickTiter™ Adenovirus Quantitation Kit	Fluorometric	20 Assays	VPK-106

Recent Product Citations

1. Triulzi, C. et al (2010). Antibody-dependent natural killer cell-mediated cytotoxicity engendered by a kinase-inactive human HER2 adenovirus-based vaccination mediates resistance to breast tumors. *Cancer Res.* **70**:7431-7441. (VPK-109)
2. Troidl, K. et al (2009). Actin-binding Rho activated protein (Abra) is essential for fluid shear stress-induced arteriogenesis. *Arterioscler. Thromb.* Vasc. Biol. **29(12)**:2093-2101. (VPK-109)
3. Hoashi, T. et al (2009). The secreted form of a melanocyte membrane-bound glycoprotein (Pmel17/gp100) is released by ectodomain shedding. *FASEB J.* 10.1096/fj.09-140921. (VPK-110)
4. Smith, M. et al (2010). PRDM1/Blimp-1 controls effector cytokine production in human NK cells. *J. Immunol.* **185**:6058-6067. (VPK-106)

Adenovirus Purification Kits

Product Name	Capacity/Prep	Size / Qty	Catalog Number
ViraBind™ Adenovirus Miniprep Kit	1 x 10^{11} VP	10 Preps	VPK-099
ViraBind™ Adenovirus Purification Kit	2.5 x 10^{12} VP	10 Preps	VPK-100

Recent Product Citations

1. Kirui, J.K. et al (2010). Gßgamma signaling promotes breast cancer cell migration and invasion. *J. Pharmacol. Exp. Ther.* **333**:393-403. (VPK-099)
2. Chen, F. et al (2011). Dynamic regulation of PDX-1 and FoxO1 expression by FoxA2 in dexamethasone-induced pancreatic ß-cells dysfunction. *Endocrinology* **152**:1779-1788. (VPK-100)
3. Prasad, S.S. et al (2011). Enzymatic activities of the human AGPAT isoform 3 and isoform 5: localization of AGPAT5 to mitochondria. *J. Lipid Res.* **52**:451-462. (VPK-100)
4. Sabbatini, M.E. et al (2010). CCK activates RhoA and Rac1 differently through G-alpha-13 and G-alpha-q in mouse pancreatic acini. *Am. J. Physiol. Cell Physiol.* **298**:C592-C605. (VPK-100)

Adenovirus Transduction Reagent

Product Name	Size / Qty	Catalog Number
ViraDuctin™ Adenovirus Transduction Reagent	10 Transductions	AD-200
	50 Transductions	AD-201

Recent Product Citations

1. Ackerman, W. et al (2008). Nuclear Factor-kappa B regulates inducible prostaglandin E synthase expression in human amnion mesenchymal cells. *Biol. Reprod.* **78**:68-76.
2. Monick, M. et al (2008). Constitutive ERK MAPK activity regulates macrophage ATP production and mitochondrial integrity. *J. Immunol.* **180**:7485-7496.

Replication-Competent Adenovirus Assay Kit

Product Name	Detection	Size / Qty	Catalog Number
Rapid RCA Assay Kit	Immunostaining	30 Assays	VPK-111

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

7758 Arjons Drive
San Diego CA 92126
info@cellbiolabs.com

Tel: 1-858-271-6500
Toll-Free: 1-888-CBL-0505
Fax: 1-858-271-6514