
Product Manual

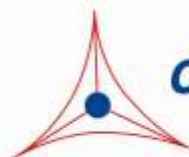
QuickTiter™ Adenovirus Titer Immunoassay Kit, Trial Size

Catalog Number

VPK-109-T

20 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Recombinant adenoviruses have tremendous potential in both research and therapeutic applications. There are numerous advantages they provide when introducing genetic material into host cells. The permissive host cell range is very wide. The virus has been used to infect many mammalian cell types (both replicative and non-replicative) for high expression of the recombinant protein. Recombinant adenoviruses are especially useful for gene transfer and protein expression in cell lines that have low transfection efficiency with liposome. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome so does not activate or inactivate host genes). Recently, recombinant adenoviruses have been used to deliver RNAi into cells.

HEK 293 cells or their variants are used as host cells for viral amplification. Recombinant adenoviruses can be grown at high titer (10^{10} VP (viral particles)/mL, which can be concentrated up to 10^{13} VP/mL) and purified by Cell Biolabs ViraBind™ Adenoviral Purification Kit or traditional CsCl ultracentrifugation.

A particular challenge in the delivery of a gene by a viral vector is the accurate measurement of virus titer. Traditionally, infectivity particles are measured in culture by a plaque-forming unit assay (PFU) that scores the number of viral plaques as a function of dilution. These methods are time-consuming (10 days), require a long infection period, and suffer from a high degree of inter-assay variability and are affected by virus-cell interactions. Cell Biolabs QuickTiter™ Adenovirus Titer Immunoassay Kit utilizes an antibody against adenovirus hexon proteins to visualize infected cells by immunocytochemistry staining. The hexon proteins are the largest and most abundant of the structural proteins in the adenovirus capsid, and they are distributed symmetrically to form capsid facets.

Cell Biolabs' QuickTiter™ Adenovirus Titer Immunoassay Kit provides a quick and complete system to functionally titer virus infectivity. This Trial Size kit provides sufficient reagents for up to 20 titrations in a 24-well plate. In contrast to the 10-day infection of a classical plaque assay, this kit only requires a 2-day infection. The kit antibody against hexon protein recognizes all 41 serotypes of adenovirus by immunocytochemistry and can be used with any adenovirus system as long as the virus is able to amplify in HEK 293 cells.

Assay Principle

Seed 293 cells in 24 or 12-well plate for 1 hr



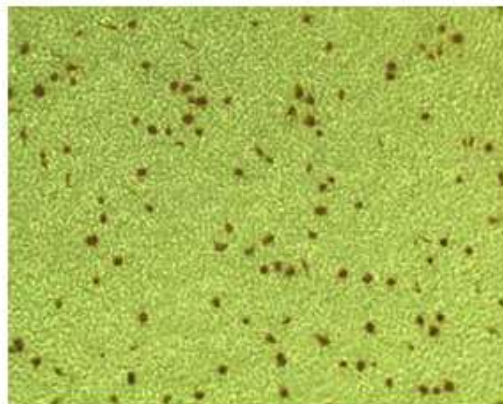
Prepare Adenovirus Serial Dilutions and Infect 293 cells for 48 hrs



Anti-Hexon Immunocytochemistry Staining



Count Positive Cells and Calculate Viral Titer



Related Products

1. AD-100: 293AD Cell Line
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent
3. VPK-106: QuickTiter™ Adenovirus Quantitation Kit
4. VPK-110: QuickTiter™ Adenovirus Titer ELISA Kit
5. VPK-111: Rapid RCA Assay Kit
6. VPK-252: RAPAd® CMV Adenoviral Expression System
7. VPK-099: ViraBind™ Adenovirus Miniprep Kit
8. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
9. VPK-120: QuickTiter™ Retrovirus Quantitation Kit

Kit Components

1. Anti-Hexon Antibody (1000X) (Part No. 10901-T): One 6 µL tube.
2. Secondary Antibody, HRP Conjugate (1000X) (Part No. 10902-T): One 10 µL tube.
3. DAB Substrate (25X) (Part No. 10903-T): One 0.5 mL tube.
4. Diluent (10X) (Part No. 10905): One 1.5 mL tube.
5. Ad-β gal Positive Control (Part No. 10904): One tube – 50 µL at 1.0×10^9 ifu/mL.

Materials Not Supplied

1. Recombinant adenovirus of interest
2. HEK 293 cells and cell culture growth medium
3. Methanol
4. 1% BSA/PBS
5. H₂O₂
6. Light Microscope
7. (optional) β-Galactosidase Staining Kit (Cat. # AKR-100)

Storage

Upon receipt, store the Ad-β-gal Positive Control at -80°C. Store all other kit components at 4°C.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

Preparation of Reagents

The table below is suggested for tests in a 24-well plate. Use twice the amount of reagents for samples in a 12-well plate.

- 1X Anti-Hexon antibody solution: Prepare a 1X anti-hexon antibody solution by diluting the provided 1000X Anti-Hexon antibody stock 1:1000 in 1% BSA/PBS. Store the diluted solution on ice.
- 1X Secondary antibody solution: Prepare a 1X Secondary antibody solution by diluting the provided 1000X stock 1:1000 in 1% BSA/PBS. Store the diluted solution on ice.
- 1X DAB working solution: Prior to use, FRESHLY prepare a 1X DAB working solution. First dilute the provided 10X Diluent to 1X with ddH₂O, and add H₂O₂ to a final concentration of 0.01%. Then dilute the 25X DAB stock to 1X with 1X Diluent/ H₂O₂ and use the 1X DAB working solution immediately.

Note: When dilute 10X diluent, use ddH₂O. Heavy metals in impure H₂O will cause DAB precipitation.

Reagents	20 tests (24-well plate)
1000X Anti-hexon Antibody	5 µL
1000X Secondary Antibody	5 µL
25X DAB	200 µL
Final Volume (Each Reagent)	5 mL

Table 1. Preparation of antibody and DAB solutions.

Preparation of Adenoviral Samples

1. Immediately before infection, create a 10-fold serial dilution of viral sample from 10⁻³ to 10⁻⁷. First, dilute original viral sample 1:100. For example, adding 10 µL of viral sample to a sterile tube containing 990 µL of culture medium.
2. Label six sterile tubes #1 to #6, and add 900 µL of culture medium to each tube. Add 100 µL of 1:100 diluted viral sample to tube #1, mix tube #1 well. Transfer 100 µL of the mixture (1:1000 dilution) to the next tube. Repeat the steps until tube #5 and use tube #6 as a blank.

Note: An Ad-β gal Positive Control is provided as an assay control, you may include 1:10⁴ and 1:10⁵ dilutions of this stock in your assay. Ad-β gal expression can also be visualized by X-gal staining.

Assay Protocol

The instructions below are suggested for assays performed in 24-well plate. Use twice as much the amount of cells and reagents for assays performed in 12-well plate.

I. Virus Infection

1. Harvest HEK 293 cells and resuspend cells in culture medium at 2.5 x 10⁵ cells/mL. Seed 1 mL in each well of a 24-well plate and incubate at 37°C, 5% CO₂ for 1 hr.

Note: Adenovirus titer assay is critically dependent of the firm attachment of cells. If the cells look thin and easy to come off during immunostaining steps, you won't get consistent result. Only use low passage 293 cells with flattened morphology or 293AD (Cat.# AD-100), a selected 293 cell line for plasmid transfection, adenovirus amplification and titrating. To improve cell adhesion, you can also precoat plate with polylysine or extracellular matrix.

2. Prepare a 10-fold serial dilution of your viral sample in culture medium. Dropwise add 100 μ L of diluted viral sample to each well of the 24-well assay plate (note: a negative control should be performed simultaneously). To ensure accuracy, perform each sample in duplicate.
3. Incubate infected cells at 37°C, 5% CO₂ for 2 days.

II. Immunostaining

1. Slowly remove medium from the wells by tilting the plate and aspirating from the edge, then fix infected 293 cells by gently adding 0.5 ml of cold methanol down the side of each well of the 24-well assay plate, taking care not to dislodge the cells. Incubate 20 minutes at -20°C.
2. Gently wash the fixed cells three times with 1X PBS, five minutes each wash.
3. Block for 1 hr with 1% BSA in PBS at room temperature on an orbital shaker.
4. Add 0.25 mL of diluted 1X anti-Hexon antibody solution to each well and incubate for 1 hr at room temperature on an orbital shaker.
5. Gently wash the fixed cells three times with 1X PBS, five minutes each wash.
6. Add 0.25 mL of diluted 1X Secondary antibody solution (HRP-conjugated) to each well and incubate for 1 hr at room temperature on an orbital shaker.
7. Gently wash the fixed cells five times with 1X PBS, five minutes each wash.
8. Add 0.25 mL of freshly diluted 1X DAB working solution to each well and incubate for 10 minutes at room temperature on an orbital shaker.

Note: Adenovirus infected cells should show dark brown staining within 5 minutes. During incubation, excess DAB starts to form light precipitates in solution, and this will not affect the staining results.

9. Aspirate DAB, wash twice with 1X PBS and add 1 mL of 1X PBS to each well.
10. Count positive stained cells (brown) for at least five separate fields per well using a light microscope and 10X objective.
11. Calculate the average number of positive cells per well and viral titer (infectious units/mL).

Example of Results

The following figures demonstrate typical titration results. One should use the data below for reference only. This data should not be used to interpret actual results.

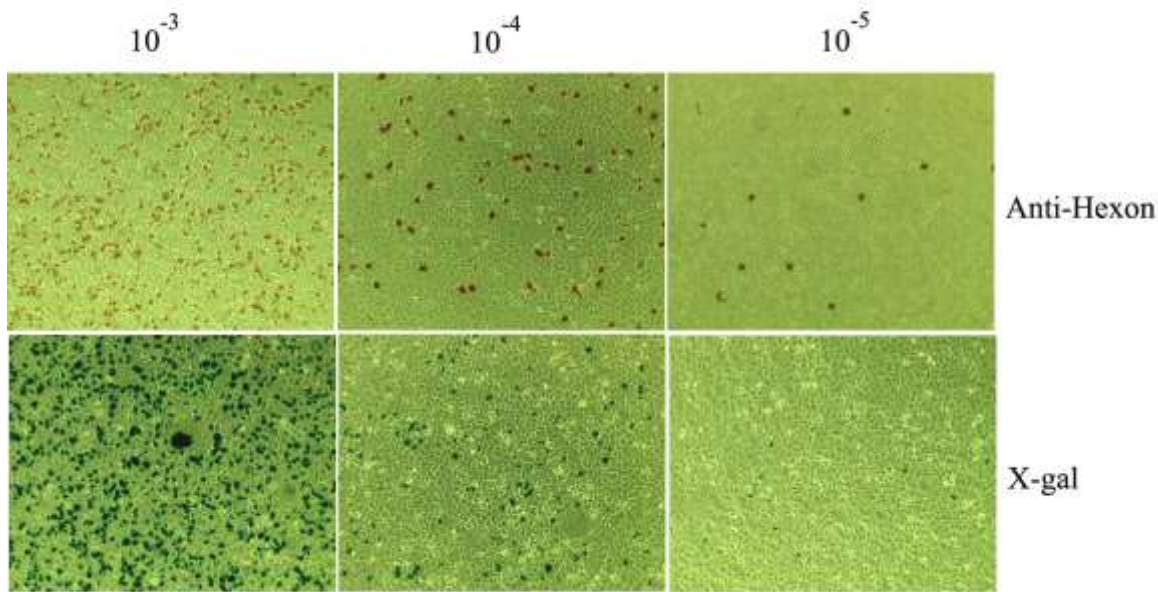


Figure 2: Ad-β gal Titration. Different dilutions of the Ad-β gal positive control were used to infect HEK 293 cells for 48 hrs. Anti-Hexon immunostaining was performed as described in Assay Instructions and X-gal staining is done by using β-Galactosidase Staining Kit (Cat. # AKR-100).

Calculation of Adenovirus Titer (Infectious Units/mL)

1. Calculate the average number of positive cells per field. Ideally, choose a dilution with 5-50 positive cells/field and count at least five fields.
2. Determine the number of fields per well

For most microscopes, a standard 10X objective lens with 10X eyepiece lens has a field diameter (D) of 1.8 mm, then:

$$\text{Area per field} = 3.14 \times (D/2)^2 = 3.14 \times 0.9^2 = 2.54 \text{ mm}^2$$

For 24-well plate, area of a well (standard 24-well plate) is 2.0 cm², therefore,

$$\text{Fields/well} = 2.0 \text{ cm}^2 / 2.54 \text{ mm}^2 = 2.0 \text{ cm}^2 / 2.54 \times 10^{-2} \text{ cm}^2 = \mathbf{79}$$

For 12-well plate, area of a well (standard 12-well plate) is 3.8 cm², therefore,

$$\text{Fields/well} = 3.8 \text{ cm}^2 / 2.54 \text{ mm}^2 = 3.8 \text{ cm}^2 / 2.54 \times 10^{-2} \text{ cm}^2 = \mathbf{150}$$

Note: If you are not sure about the field diameter of the 10X objective lens you are using or you are using objective lenses other than 10X, the field diameter can be determined by aligning with the grids of hemacytometer (Figure 2), or referring to Table 2.

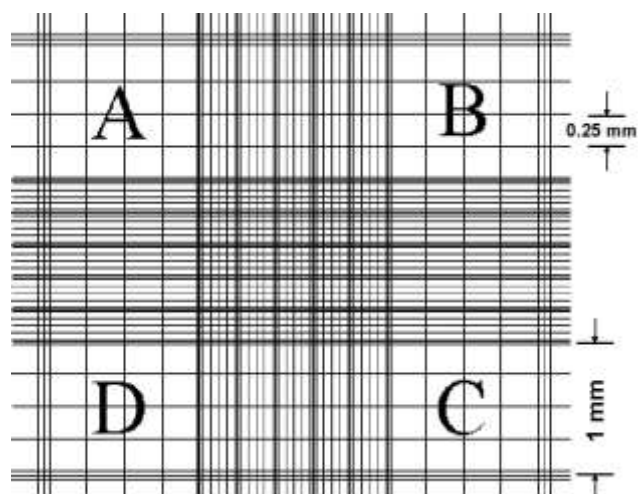


Figure 2. Hemacytometer Grid Dimensions.

Objective Lenses	Eyepiece Lenses (10X)			Fields/Well	
	Total Magnification	Field Diameter	Field Area (mm ²)	12-well Plate	24-well Plate
4X	40X	5 mm	19.6	19	10
10X	100X	1.8 mm	2.54	150	79
20X	200X	0.9 mm	0.64	594	313

Table 2. Field sizes of objective lenses.

3. Calculate viral titer (Infectious Units or ifu/mL)

Tests in 24-well:

$$\text{Viral Titer (ifu/mL)} = \frac{(\text{average positive cells/field}) \times (79 \text{ fields/well}) \times (\text{dilution factor})}{(0.1 \text{ mL})}$$

Tests in 12-well:

$$\text{Viral Titer (ifu/mL)} = \frac{(\text{average positive cells/field}) \times (150 \text{ fields/well}) \times (\text{dilution factor})}{(0.1 \text{ mL})}$$

Calculation Example:

A serial of 10-fold dilutions of the provided Ad-β gal Positive Control was made and its titer was determined in a 24-well plate as described in assay instruction. Ten fields were counted and the average positive cells/field is 12 for 1/10⁵ dilution under a standard 10X objective, therefore:

$$\text{Viral Titer (ifu/mL)} = \frac{(\text{average positive cells/field}) \times (79 \text{ fields/well}) \times (\text{dilution factor})}{(0.1 \text{ mL})}$$

$$\text{Viral Titer (ifu/mL)} = \frac{(12/\text{field}) \times (79 \text{ fields/well}) \times (10^5)}{(0.1 \text{ mL})} = 0.95 \times 10^9 \text{ (ifu/mL)}$$

References

1. Bewig, B., and W. E. Schmidt (2000) Accelerated titering of adenoviruses. *BioTechniques* **28**:870-873.

Recent Product Citations

1. McQuerry, J.A. et al. (2021). Tepoxalin increases chemotherapy efficacy in drug-resistant breast cancer cells overexpressing the multidrug transporter gene ABCB1. *Transl Oncol.* **14**(10):101181. doi: 10.1016/j.tranon.2021.101181.
2. Tournier, B.B. et al. (2021). Amyloid and Tau Induce Cell Death Independently of TSP0 Polymerization and Density Changes. *ACS Omega.* doi: 10.1021/acsomega.1c01678.
3. Oliveira, E.R.A. et al (2021). Intracellular Sequestration of the NKG2D Ligand MIC B by Species F Adenovirus. *Viruses.* **13**(7):1289. doi: 10.3390/v13071289.
4. Borrás, T. et al. (2020). Generation of a Matrix Gla (Mgp) floxed mouse, followed by conditional knockout, uncovers a new Mgp function in the eye. *Sci Rep.* **10**(1):18583. doi: 10.1038/s41598-020-75031-7.
5. Wei, W.Z. et al. (2020). Diversity Outbred Mice Reveal the Quantitative Trait Locus and Regulatory Cells of HER2 Immunity. *J Immunol.* doi: 10.4049/jimmunol.2000466.
6. Robles-Rodríguez, O.A. et al. (2020). Antitumor effect of adenoviruses expressing mutant nononcogenic E7 versions from HPV-16 fused to calreticulin. *JBUON.* **25**(1): 543-548.
7. Rollier, C.S. et al. (2020). Modification of Adenovirus vaccine vector-induced immune responses by expression of a signalling molecule. *Sci Rep.* **10**(1):5716. doi: 10.1038/s41598-020-61730-8.
8. Lokhandwala, S. et al. (2019). Adenovirus-vectored African Swine Fever Virus antigen cocktails are immunogenic but not protective against intranasal challenge with Georgia 2007/1 isolate. *Veterinary Microbiology.* **235**:10-20. doi: 10.1016/j.vetmic.2019.06.006.
9. Sang, Y. et al. (2019). Ileal transcriptome analysis in obese rats induced by high-fat diets and an adenoviral infection. *Int J Obes (Lond).* doi: 10.1038/s41366-019-0323-2.
10. Hao, J. et al. (2018). Prostate Cancer-Specific of DD3-driven Oncolytic Virus-harboring mK5 Gene. *Open Med (Wars).* **14**: 1–9. doi: 10.1515/med-2019-0001.
11. Mao, L.J. et al. (2018). Oncolytic Adenovirus Harboring Interleukin-24 Improves Chemotherapy for Advanced Prostate Cancer. *J Cancer.* **9**(23):4391-4397. doi: 10.7150/jca.26437.
12. Konishi, K. et al. (2018). In vitro approach to elucidate the relevance of carboxylesterase 2 and N-acetyltransferase 2 to flupirtine-induced liver injury. *Biochem Pharmacol.* **155**:242-251. doi: 10.1016/j.bcp.2018.07.019.
13. Que, W. et al. (2018). NK4 inhibits the proliferation and induces apoptosis of human rheumatoid arthritis synovial cells. *Cell Biochem Funct.* **36**(5):273-279. doi: 10.1002/cbf.3339.
14. Alharbi, N.K. et al. (2017). ChAdOx1 and MVA based vaccine candidates against MERS-CoV elicit neutralising antibodies and cellular immune responses in mice. *Vaccine* **35**(30):3780-3788.
15. Xu, Y. et al. (2017). RGD-modified oncolytic adenovirus-harboring shPKM2 exhibits a potent cytotoxic effect in pancreatic cancer via autophagy inhibition and apoptosis promotion. *Cell Death Dis.* **8**(6):e2835.
16. Yuan, S. et al. (2017). An oncolytic adenovirus expressing SNORD44 and GAS5 exhibits anti-tumor effect in colorectal cancer cells. *Hum. Gene Ther.* doi:10.1089/hum.2017.041.
17. Lokhandwala, S. et al. (2017). Adenovirus-vectored novel African Swine Fever Virus antigens elicit robust immune responses in swine. *PLoS One* **12**(5):e0177007.

18. Sugase, T. et al. (2017). Suppressor of cytokine signaling-1 gene therapy induces potent antitumor effect in patient-derived esophageal squamous cell carcinoma xenograft mice. *Int J Cancer*. doi: 10.1002/ijc.30666.
19. Keshava, S. et al. (2016). Intrapleural adenoviral-mediated endothelial cell protein C receptor gene transfer suppresses the progression of malignant pleural mesothelioma in a mouse model. *Sci. Rep.* **6**:36829.
20. Xiong, X. et al. (2016). SIRT6 protects against palmitate-induced pancreatic β -cell dysfunction and apoptosis. *J. Endocrinol.* **231**:159-165.
21. Lokhandwala, S. et al. (2016). Induction of robust immune responses in swine using a cocktail of adenovirus-vectored african swine fever virus antigens. *Clin Vaccine Immunol.* doi:10.1128/CVI.00395-16.
22. Ansari, S. A. et al. (2016). The role of putative phosphatidylserine-interactive residues of tissue factor on its coagulant activity at the cell surface. *PLoS One*. doi: 10.1371/journal.pone.0158377.
23. Martin, L. J. & Wong, W. (2016). Enforced DNA repair enzymes rescue neurons from apoptosis induced by target deprivation and axotomy in mouse models of neurodegeneration. *Mech Ageing Dev.* doi: 10.1016/j.mad.2016.06.011.
24. Gibot, L. et al. (2015). Cell-based approach for 3D reconstruction of lymphatic capillaries in vitro reveals distinct functions of HGF and VEGF-C in lymphangiogenesis. *Biomaterials*. doi: 10.1016/j.biomaterials.2015.11.027.
25. Herath, S. et al. (2015). Analysis of T cell responses to chimpanzee adenovirus vectors encoding HIV gag-pol-nef antigen. *Vaccine*. doi: 10.1016/j.vaccine.2015.10.111.
26. Yang, Y. et al. (2015). RGD-modified oncolytic adenovirus exhibited potent cytotoxic effect on CAR-negative bladder cancer-initiating cells. *Cell Death Dis.* **6**:e1760.
27. Nakao, S. et al. (2015). Stimulus-dependent regulation of nuclear Ca²⁺ signaling in cardiomyocytes: a role of neuronal calcium sensor-1. *PLoS One.* **10**:e0125050.
28. Patsouris, D. et al. (2014). Insulin resistance is associated with MCP1-mediated macrophage accumulation in skeletal muscle in mice and humans. *PLoS One.* **9**:e110653.
29. Xiong, Y. et al. (2014). Long term exposure to L-arginine accelerates endothelial cell senescence through arginase-II and S6K1 signaling. *Aging (Albany NY).* **6**:369.
30. Barrett, A. et al. (2014). A crucial role for DOK1 in PDGF-BB-stimulated glioma cell invasion through p130Cas and Rap1 signalling. *J Cell Sci.* **127**:2647-2658.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2013-2021: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.