

## RhoA N19 Recombinant Adenovirus (Dominant Negative)

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**CATALOG NUMBER:** ADV-156      **STORAGE:** -80°C

**QUANTITY AND CONCENTRATION:** 50 µl,  $1 \times 10^{11}$  VP/mL in TBS containing 10% Glycerol

### **Background**

Recombinant adenoviruses have tremendous potential in both research and therapeutic applications. There are numerous advantages in using an adenovirus to introduce 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.

Three members of the Rho family small GTPase, Rho, Rac, and Cdc42, have been shown to play a crucial role in regulating the organization of the actin cytoskeleton in response to extracellular stimuli. Activation of Rho, Rac, and Cdc42 in quiescent Swiss 3T3 fibroblasts induces the assembly of filamentous actin into stress fibers, lamellipodia, and filopodia, respectively. In addition to these effects on the actin cytoskeleton, it has been shown Rac and Cdc42 (and in some cells Rho) can activate JNK and p38 that leads to transcriptional activation. In fibroblast cells, Rho, Rac, and Cdc42 have each been implicated in cell cycle control. The provided recombinant adenovirus contains dominant negative form of human RhoA (T19N).

### **Safety Consideration**

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

### **Methods**

The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. If not enough virus is used, it will not give 100% of infection. If too much virus is used, it will cause cytotoxicity or other undesired effects. The amount of adenovirus cell surface receptors vary greatly among different cell types therefore the optimal concentration differs dramatically between cell types. A range of 10-200 MOI (multiplicity of infection) is used for most cell lines, but up to 1000 MOI may be used for lymphoid cell lines.

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. In contrast to the 10-day infection of a classical plaque assay, Cell Biolabs' QuickTiter™ Adenovirus Titer Immunoassay Kit (Cat. #VPK-109) only requires 2-day infection, and there is no agar overlay step. The kit antibody against hexon protein recognizes all serotypes of adenovirus by immunocytochemistry (see Flow Chart).

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



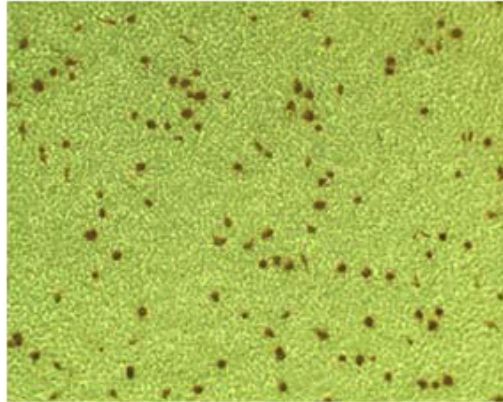
Prepare Adenovirus Serial Dilutions  
and Infect 293 cells for 48 hrs



Anti-Hexon Immunocytochemistry Staining



Count Positive Cells and Calculate Viral Titer



### References

1. Bett AJ, Haddara W, Prevec L and Graham FL. (1994) *Proc Natl Acad Sci U S A.* **91**:8802-6.
2. Robbins, P. D., Tahara, H., and Ghivizzani, S. C. (1998) *Trends Biotechnol.* **16**:35-40.
3. Huang, S., Stupack, D., Mathias, P., Wang, Y., and Nemerow, G. (1997) *Proc. Natl. Acad. Sci. U S A.* **94**:8156-8161.
4. Bergelson, J. M., J. A. Cunningham, G. Droguett, E. A. Kurt-Jones, A. Krithivas, J. S. Hong, M. S. Horwitz, R. L. Crowell, and R. W. Finberg. (1997) *Science* **275**:1320-1323.
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### Recent Product Citations

1. Wang, Q. et al. (2016). Role of moesin in advanced glycation end products-induced angiogenesis of human umbilical vein endothelial cells. *Sci Rep.* doi:10.1038/srep22749.
2. Neal, M. et al. (2013). A critical role for TLR4 induction of autophagy in the regulation of enterocyte migration and the pathogenesis of necrotizing enterocolitis. *J. Immunol.* **190**:3541-3551.
3. Black, S.A. et al. (2008). TGF $\beta$ 1 stimulates connective tissue growth factor (CCN2/CTGF) expression in human gingival fibroblasts through a RhoA-independent, Rac1/Cdc42-dependent mechanism: statins with forskolin block TGF $\beta$ 1-induced CCN2/CTGF expression. *J. Biol. Chem.* **283**:10835-10847.
4. Perez-Moreno, M. et al. (2008). Loss of p120 catenin and links to mitotic alterations, inflammation, and skin cancer. *Proc. Natl. Acad. Sci. U S A* **105**:15399-15404.

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