PAK1 T423E Recombinant Adenovirus

CATALOG NUMBER: ADV-204 **STORAGE:** -80ºC

QUANTITY AND CONCENTRATION: 50 µl, 1 x 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.

Regulation of cell growth, differentiation, and survival requires the coordinated modulation of multiple cell signaling pathways. The signals from the small GTPase Ras are among the best characterized signaling pathways. Ras binds and activates several effectors including Raf, phosphatidylinositol (PI) 3-kinase and Ral GDS. PI-3 kinase generates phospholipid second messengers that stimulate downstream signaling pathways to regulate the actin cytoskeleton and also promote cell survival by inhibiting apoptosis. PI-3 kinase regulates the actin cytoskeleton through the small GTPase Rac. The p21-activated protein kinases (PAKs) are serine-threonine protein kinases identified because they bind and are activated by the small GTPases Rac and Cdc42. Expression of PAK in cells stimulates some of the changes in the actin cytoskeleton associated with Rac and Cdc42, including stimulation of cell ruffling and inhibition of stress fibers. It has been shown that PAKs also transducer signals to MAPKs to regulate cell growth and differentiation. The provided recombinant adenovirus contains constitutively active form of human PAK1 (T423E), the mutant has strong kinase activity and bind Rac/CDC42 normally.

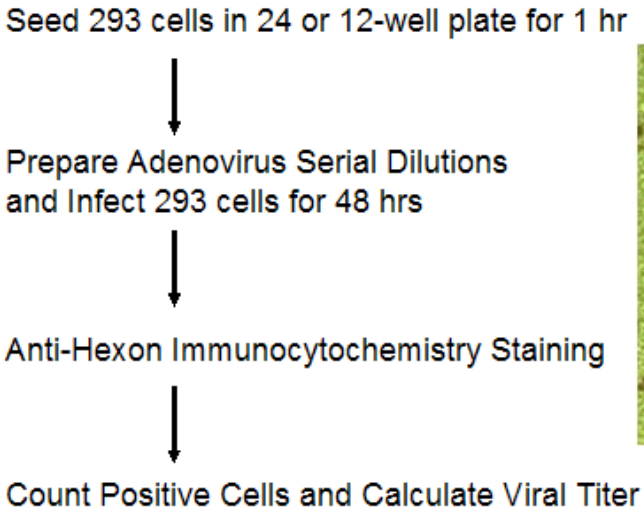
Safety Consideration

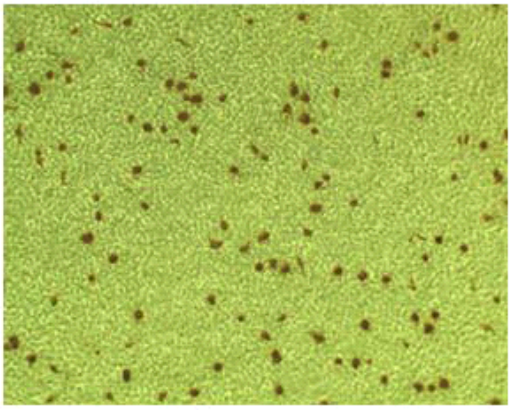
Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

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).





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.
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Recent Product Citation

1. Nie, J. et al. (2013). SAD-A Kinase Controls Islet β-cell Size and Function as a Mediator of mTORC1 Signaling. *PNAS*. **110**:13857-13862.
2. Nie, J. et al. (2012). Synapses of Amphids Defective (SAD-A) Kinase Promotes Glucose-stimulated Insulin Secretion through Activation of p21-activated Kinase (PAK1) in Pancreatic β-Cells. *J. Biol. Chem*. **287**: 26435-226444.

Warranty

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