shAKT1 Recombinant Adenovirus

CATALOG NUMBER: ADV-417 **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.

The AKT /protein kinase B was identified as a serine/threonine protein kinase with high homology with the protein kinases A and C. At the same time, this kinase was identified as the cellular homologue of the viral oncoprotein v-AKT. AKTs contain an N-terminal Pleckstrin homology domain, followed by a kinase domain and a C-terminal regulatory tail. AKT is an important regulator of various cellular processes including glucose metabolism, cell survival, and angiogenesis. The phosphoinositide 3-kinase and its product phosphoinositide-3,4,5-triphosphate can promote translocation of AKT to the plasma membrane and the phosphorylation at the two sites, Thr-308 and Ser-473. The activated AKT then phosphorylates substrates including glycogen synthase kinase-3, Bad, eNOS, caspase-9, and forkhead transcription factors.

The provided recombinant adenovirus contains a H1 promoter driven shAKT1 specific targeting at the 3' untranslated sequence of mouse AKT1.

Safety Consideration

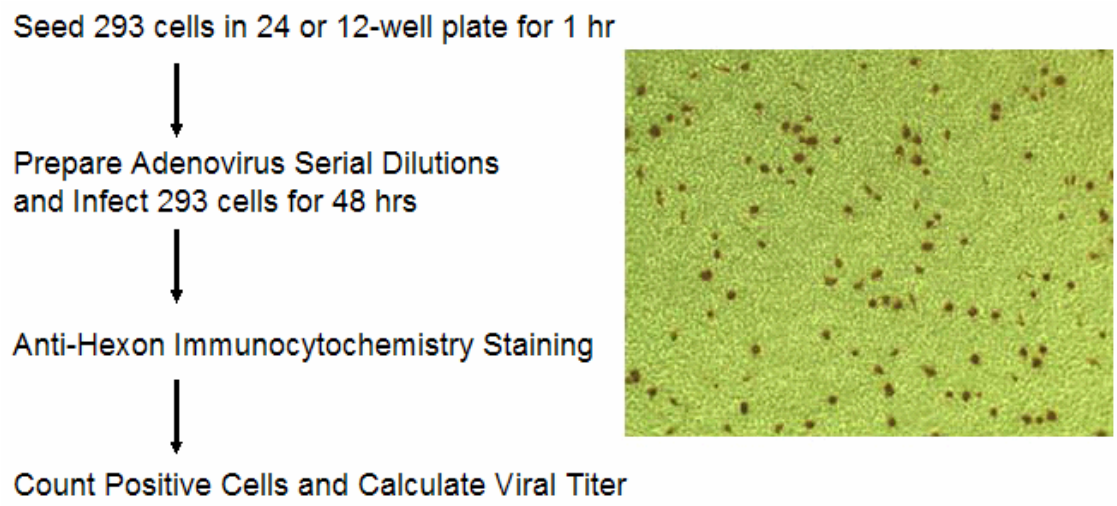
Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

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.
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.
5. Marshall C. J., Lloyd A. C., Morris J. D. , Paterson H., Price B and Hall A. (1989) *Int J Cancer Suppl.* 4:29-31.

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