Product Data Sheet

PKC-α Recombinant Adenovirus (Dominant Negative)

CATALOG NUMBER: ADV-410 **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.

Protein kinase C (PKC) is a multigene family that encodes at least 11 distinct isoforms of lipid-regulated serine/threonine kinases. Specific isoforms play pivotal roles in several signal transduction pathways that regulate cellular growth, transformation, and differentiation. The isoforms are classified into three groups, based on their structure and cofactor requirement: (i) classic PKCs (α, βI, βII, and γ), which are activated by diacylglycerol (DAG) or calcium, (ii) novel PKCs (δ, ε, η, θ, and µ), which are activated by DAG but not by calcium, and (iii) atypical PKCs (ζ and ι), which are not responsive to either DAG or calcium. Each of these isoforms contains an N-terminal regulatory domain and a C-terminal catalytic kinase domain. The regulatory domains contain a pseudosubstrate domain, an autoinhibitory domain with substrate-like sequences that maintain the enzyme in an inactive state presumably by interacting with the substrate binding site in the catalytic domain. PKC activators like DAG, phorbol esters, and calcium are thought to relieve this intramolecular inhibition, resulting in a conformational change that liberates the substrate binding domain from the pseudosubstrate domain, thereby activating the enzyme. The provided recombinant adenovirus contains a dominant negative PKC-α with a K/R point mutation at the ATP binding site of Bovine PKC-α sequence.

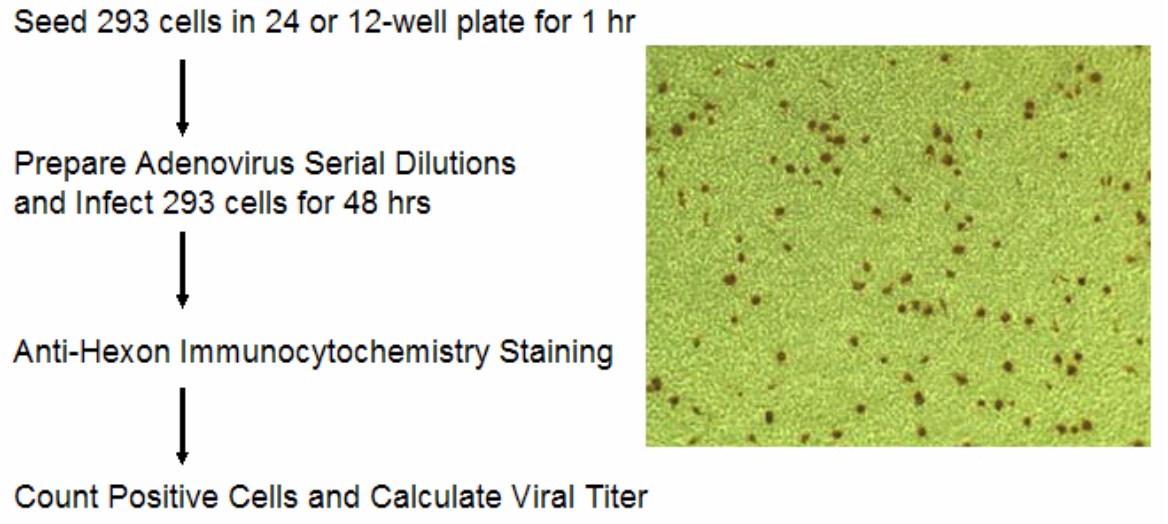
Safety Consideration

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. Newton, A. C. (1995) *J. Biol. Chem.* **270,** 28495–28498
2. Soh, J and Weinstein I. B. (2003) *J. Biol. Chem.* **278,** 34709-34716

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