Product Data Sheet

IκB-α S32A Recombinant Adenovirus (Dominant Negative)

CATALOG NUMBER: ADV-302 **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.

NF-κB is a multi-subunit transcription factor that is involved in the regulation of a large number of genes that control various aspects of the immune and inflammatory response. NF-κB/Rel complex is bound and inhibited by IκB proteins. Treatment of cells with proinflammatory cytokines results in the phosphorylation of the IκB proteins, that leads to further ubiquitination and degradation by the ubiquitin-26S proteasome pathway. The active NF-κB is liberated from IκB and subsequently translocates to the nucleus, there to activate transcription of its target genes. The provided recombinant adenovirus contains the dominant negative form of human IκB-α S32A mutant. The phosphorylation-defective IκBα (S32A) acts by sequestering the cytoplasmic NFκB pool in a manner that is insensitive to extracellular stimuli.

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

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Prepare Adenovirus Serial Dilutions and Infect 293 cells for 48 hrs

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Anti-Hexon Immunocytochemistry Staining

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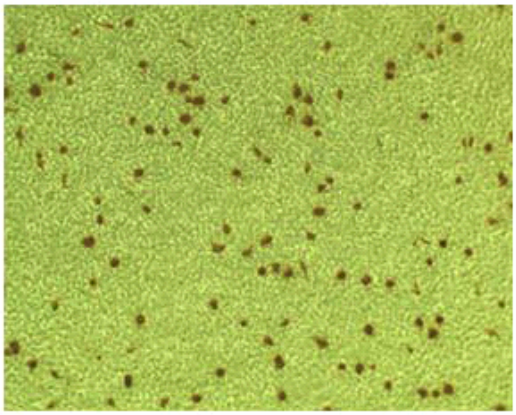
Count Positive Cells and Calculate Viral Titer



References

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2. Robbins, P. D., Tahara, H., and Ghivizzani, S. C. (1998) *Trends Biotechnol.* 16, 35-40.
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Recent Product Citations

1. Ackerman, W. et al. (2007). Nuclear Factor-kappa B regulates inducible prostaglandin E synthase expression in human amnion mesenchymal cells. *Biol. Reprod.* 78:68-76.
2. Martin, A.P. et al. (2008). Lapatinib resistance in HCT116 cells is mediated by elevated MCL-1 expression and decreased BAK activation and not by ERBB receptor kinase mutation. *Mol. Pharmacol.* **74**:807-822.
3. Johnston, R.K. et al. (2009). ß3-integrin mediated ubiquitination activates survival signaling during myocardial hypertrophy. *FASEB J.* 10.1096/fj.08-127480.

Warranty

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