MEK5 Recombinant Adenovirus (Dominant Negative)

CATALOG NUMBER: ADV-130 **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.

Mitogen-activated protein kinases (MAPK), including ERK1/2, p38, and JNK1/2, are important regulators of cell function. The ERK MAPKs are most frequently activated by mitogens, whereas the JNK and p38 MAPKs are strongly responsive to stress and inflammatory signals. The extracellular signal-regulated kinases (ERKs), are activated in response to growth factors, via the Ras proto-oncogene and multiple intracellular kinase phosphorylation cascade events. ERK5, also known as BMK1, shares high homology in the N-terminal kinase domain with ERK1/2, but has a unique long C-terminal domain. The activity of ERK5 is up-regulated through the phosphorylation of the TEY activation motif by MEK5. The provided recombinant adenovirus contains dominant negative form of human MEK5 sequence with three copies of HA epitope at C-terminal. The MEK5 (A) mutant cannot be phosphorylated, since the dual phosphorylation site S311/S315 has been changed to A311/A315.

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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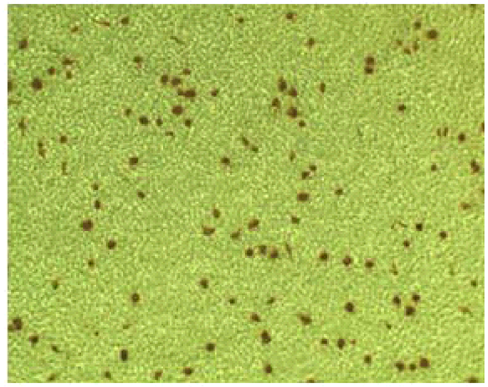
Count Positive Cells and Calculate Viral Titer



References

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Recent Product Citation

1. Wu, Y. et al. (2012). ERK5 Regulates Glucose-Induced Increased Fibronectin Production in the Endothelial Cells and in the Retina in Diabetes. *Invest. Opthamol. Vis. Sci*. **53**: 8405-8413.

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