MEKK1 Recombinant Adenovirus

CATALOG NUMBER: ADV-135 **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 MAPKs are activated through multiple intracellular phosphorylation cascade events. The core unit includes MAPKKKs and MAPKKs. MEKK1 is regulated by Nck and germinal center kinase (GCK), as well as the scaffolding protein Traf2, primarily through interactions with its amino terminus. Other binding partners include: JNK, MKK4, Tax, one of the transforming proteins produced by HTLV-1, 14-3-3 proteins, Raf-1 p115 RhoGAP, Rac and Cdc42 small GTPases. It has been proposed that MEKK1 can act as the scaffold of its own MAP kinase signaling module in a manner reminiscent of Ste5p of the yeast pheromone-signaling pathway. The provided recombinant adenovirus contains human MEKK1 sequence.

Safety Consideration

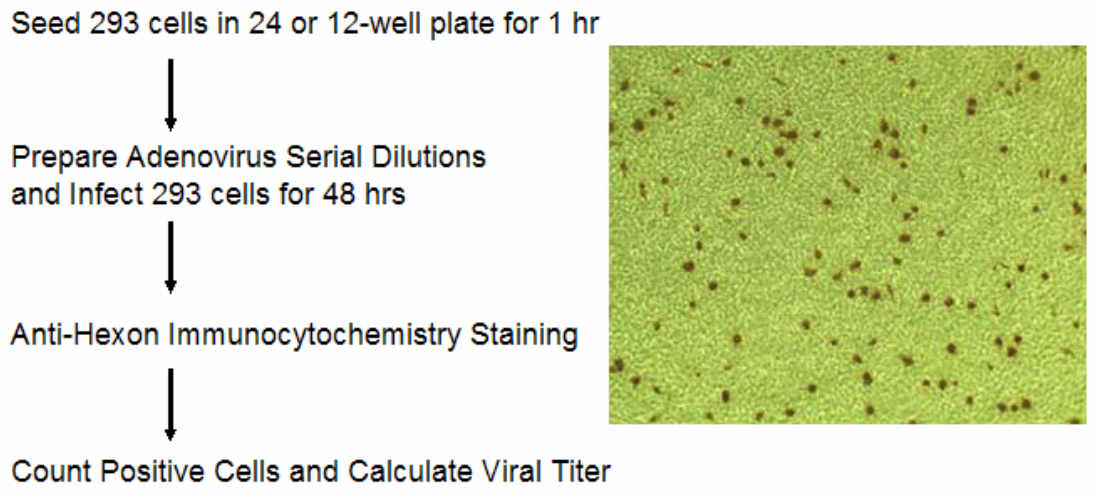
Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

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

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com