Product Data Sheet

Ras V12 Recombinant Adenovirus (Constitutively Active)

CATALOG NUMBER: ADV-146 **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.

Ras genes encode 21 kDa guanine nucleotide-binding proteins, including H-, K- and N-Ras. H-Ras was first identified as oncogene, mutated Ras genes have been found in many human tumors. Like all GTPases, Ras act as molecular switch to control downstream cellular events. The interconversion of the inactive GDP-bound form into the active GTP-bound form is regulated by guanine nucleotide exchange factors, whereas inactivation of the GTP-bound form is stimulated by GTPase-activating proteins (GAPs). Ras in its active GTP bound form binds to Raf, resulting in activation of MAP kinase cascade. The provided recombinant adenovirus contains constitutively active form of human H-Ras (G12V).

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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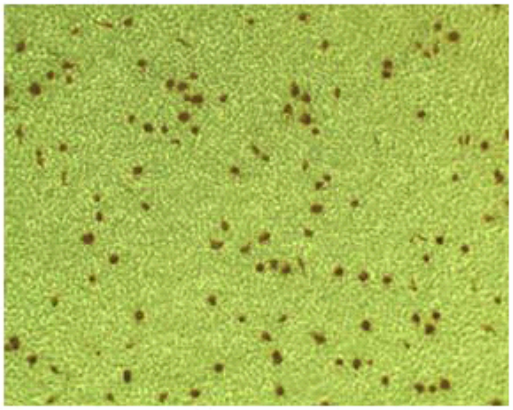
Count Positive Cells and Calculate Viral Titer



References

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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