

Carbonic Anhydrase 9 Recombinant Adenovirus

CATALOG NUMBER: ADV-602

STORAGE: -80°C

QUANTITY AND CONCENTRATION: 50 µl, 1×10^{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.

Numerous human tumor-associated antigens (Ags) have been identified, either by screening cDNA libraries with sera derived from cancer patients containing an antibody (Ab) to a tumor-associated Ag (SEREX) or by using T lymphocytes specific for tumor peptides presented in the context of specific HLA alleles. Carbonic anhydrase 9 (CA9) is a transmembrane protein overexpressed in a wide variety of tumor types and induced by hypoxia. Focal expression of CA9 occurs in >90% of carcinomas of the cervix, digestive tract, head and neck, as well as glioblastomas and basal cell carcinomas. CA9 expression has been used to distinguish between malignant and preneoplastic lesions of the lung and cervix. Recently, CA9 expression was found to be a significant predictor of disease-specific and metastasis-free survival in patients with locally advanced squamous cell carcinoma of the uterine cervix, after allowing for stage, age, and tumor grade. The provided recombinant adenovirus contains human Carbonic anhydrase 9 (CA9).

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



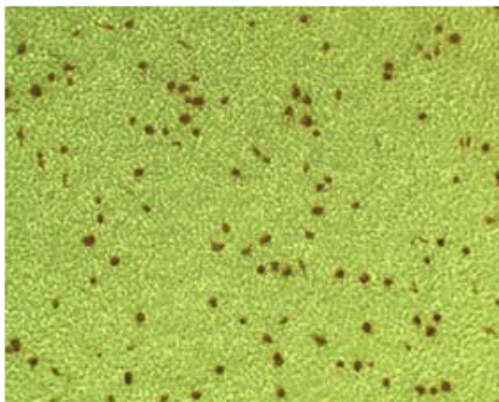
Prepare Adenovirus Serial Dilutions
and Infect 293 cells for 48 hrs



Anti-Hexon Immunocytochemistry Staining



Count Positive Cells and Calculate Viral Titer



References

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