Enhanced Adenovirus Safety Features: Replication Incompetency

Introduction and Principle

For transient gene expression and recombinant protein production using adenovirus, it is very important to minimize the risk of generating replication-competent adenovirus in order to contain the virus for laboratory safety. Ready-to-use adenovirus provided by **abm** is based on human adenovirus serotype 5 (Ad5) rendered replication-incompetent.

This is accomplished by producing 2nd generation adenoviral constructs that omit the early viral genes, E1 and E3, but still maintain the necessary viral packaging signals and inverted terminal repeat sequences required for viral transcription. E1A is a viral transcription factor required for induction of early genes necessary for viral replication, progression of the cell cycle, and inhibition of apoptosis. E3 plays an important role in suppression of host immunity. A replication-incompetent (-E1/-E3) virus can therefore efficiently infect cells but, once the infection has taken place, it cannot produce new virions, unless amplified in packaging cell lines that express E1 gene products (such as HEK293 cells).

Quality Control

All ready-to-use adenovirus from **abm** is tested for replication-incompetency following production. After adenoviral transduction of target cells, the cells are tested for the presence of E1A gene using **abm**'s One-Step RT-PCR Kit (**Cat. No. G174**). Since E1A gene is necessary for viral replication, its presence can be used to determine the replication competency of the adenovirus.

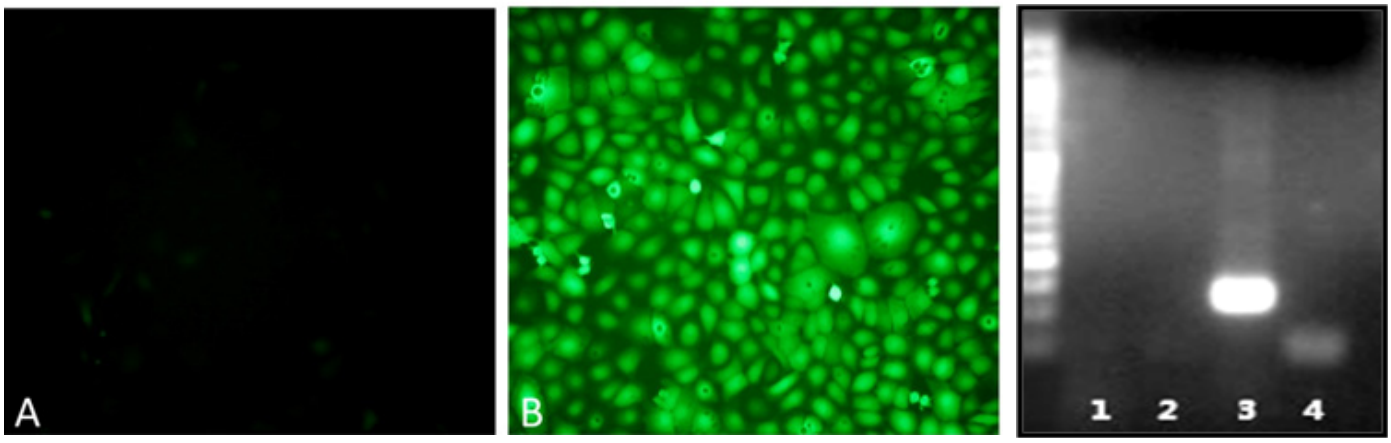

Figure 1. GFP Adenovirus (Cat. No. 000541A) transduction efficiency and replication competency is evaluated using SKOV3-IP cells. (A) SKOV3-IP cells without adenoviral transduction and (B) SKOV3-IP cells 24 hours post-transduction with GFP Adenovirus, viewed under a fluorescence microscope. (C) Cells from A and B are tested for the presence of E1A gene using RT-PCR. Lane 1: SKOV3-IP cells. Lane 2: SKOV3-IP cells transduced with GFP Adenovirus. Lane 3: Positive control (HEK293 cells). Lane 4: No-Template Control for PCR.

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