Manufacturing of RCA-free adenoviral vectors

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Adenoviral vectors are frequently used as delivery tools for vaccine and gene therapy applications. Once such vectors have infected a target cell, it is of utmost importance that they do not spread further in a patient's body. Therefore, avoiding the occurrence and further propagation of replication-competent adenovirus (RCA) during production is crucial. Since the risk of RCA occurrence depends on the adenoviral vector and the cell line used for virus stock generation and manufacturing, selecting an appropriate cell line and production platform is critical.

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ADVANTAGES OF ADENOVIRAL VECTORS FOR MEDICAL APPLICATIONS

Adenovirus is an intensively studied vector offering simple vector construction, efficient transduction and high stability, which enables simple handling and long-term storage. Adenoviral vectors are increasingly being developed for both gene therapy and vaccine applications. Focusing on vaccines in particular, adenoviral vectors offer a number of advantages including the stimulation of a robust immune response, and the fact that the use of a strong promoter can result in more persistent duration of antigen expression when compared to vaccines based on recombinant protein or inactivated viruses. In addition, recently-approved adenoviral vaccines for COVID-19 represent a strong precedent for the high suitability of these vectors.

IMPORTANCE OF AVOIDING RCA IN THE MANUFACTURING PROCESS

The formation of RCA is highly undesirable during vaccine and biotherapeutic development, as it can trigger adverse immune responses and spread in the human body. Consequently, regulators such as FDA and EMA have issued guidance on maximum levels of RCA. Preparations must be tested for RCA and discarded if they exceed the allowed threshold, which can lead to significant additional cost and delays in clinical development and supply to the market.

ACHIEVING RCA-FREE PRODUCTION BY DESIGN WITH THE CAP® AD PLATFORM

Usually the early region 1 (E1) in the viral genome, which is necessary for replication, has been deleted in adenoviral vectors, resulting in so-called AdΔE1 vectors. In order to produce a vector that lacks an essential component for its own replication, a complementary cell line that carries a stable insertion of the E1 region is needed. However, common cell lines such as HEK 293 carry the risk of spontaneous RCA formation due to the presence of two large homology regions and the resulting probability of homologous recombination **(Figure 1)**.

The CAP Ad platform has been specifically designed to address this as CAP cells contain only one very short and additionally inverted homology region, thereby minimizing risk of RCA formation **(Figure 1)**.

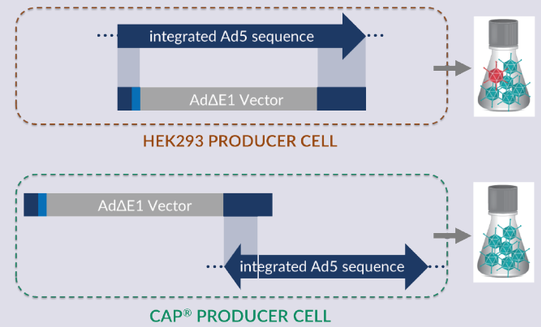
INDUSTRIAL SCALE MANUFACTURE WITH THE CAP AD PLATFORM

CAP cells are an ideal producer cell line for the development of biotherapeutics. The cell line is derived from human amniocytes, fully documented and available as a GMP bank. A Biologics Master File has been deposited with the FDA and can be referenced.

CAP cells can be cultivated in all common forms and scales of bioreactors using serum-free, chemically defined media and cell densities reach up to 15–20 million cells/mL in fed-batch, with high viabilities and productivities.

In summary, the CAP Ad platform provides a unique solution for industrial scale manufacture of adenoviral vectors at high titers, while minimizing the risk of RCA formation and therefore complying with recent regulatory standards **(Figure 2)**.

Figure 1. RCA-free production by design in CAP cells. CAP cells contain only one very short and inverted region of homology to the AdΔE1 vector. No RCAs were detected in CAP cell derived material.



Cell Line	RCA in $5x10^{10}$ VP
HEK293	5-500
CAP®	None

Courtesy of SIRION BIOTECH GmbH (part of PerkinElmer)

Figure 2. CAP Ad platform: Industrial scale manufacturing with minimized risk for RCA formation throughout the entire manufacturing process, from primary Ad stock to large scale GMP-production.