

Simplify adenovirus production with a simple and rapid titer test

Post-transfection—Determine if infectious virus was produced, even before cytopathic events are observed

Post-amplification—Ensure high titer by harvesting amplified adenovirus at the optimal time

Introduction

The Adeno-X GoStix protocol takes as little as 30 seconds to indicate the presence of adenovirus particles in your post-transfection or post-amplification supernatant. Adeno-X GoStix are designed to instantly confirm the presence of adenovirus in cell culture supernatants or lysates. The test detects adenoviral hexon proteins and can be used to determine if infectious virus was produced post-transfection, or to select the best time to harvest your virus. The included Hexon Control provides confirmation of GoStix test function (Figure 1).

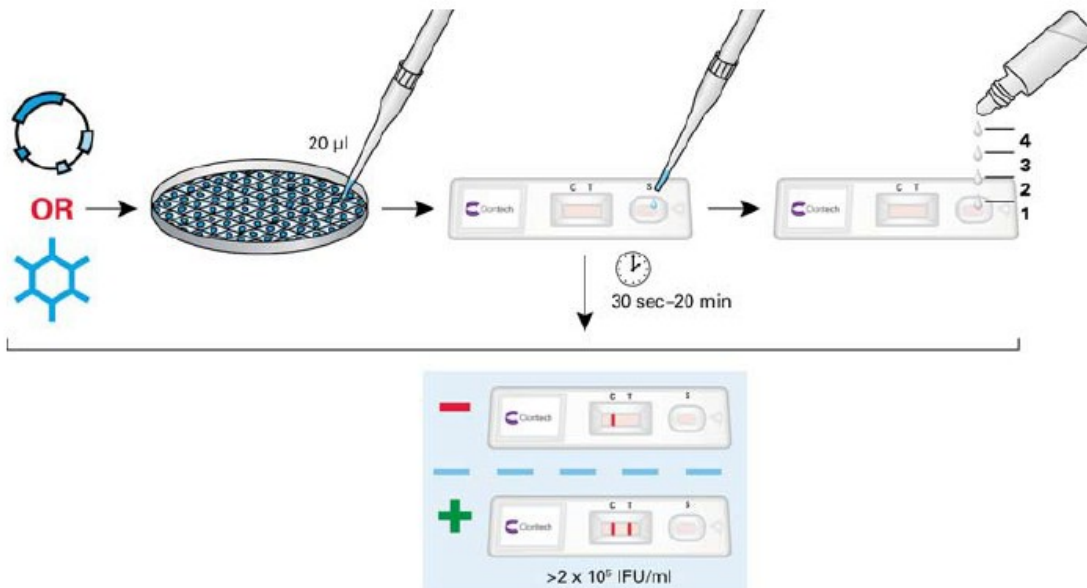


Figure 1. The Adeno-X GoStix workflow. The Adeno-X GoStix protocol takes only a couple of minutes to indicate the presence of adenovirus particles. First, supernatant from transfected cells is placed on the cassette, followed by 4 drops of chase buffer. If the viral titer in your supernatant is $>2 \times 10^6$ IFU/ml you will detect a band in the test window within minutes.

Results

Early detection of adenovirus during rescue

Adeno-X GoStix were used to confirm virus production during the rescue procedure. PacI-digested adenovirus expressing ZsGreen1 was transfected into HEK 293 cells, which were monitored for the emergence of cytopathic effects by phase and fluorescence microscopy. Twenty microliters of culture medium was applied to the Adeno-X GoStix on each day. Although no virus was detected at the start of the experiment, at four days post-transfection, Adeno-X GoStix were able to detect virus in culture supernatants containing plaques that were only weakly perceptible under phase microscopy and confirmed by fluorescence microscopy (Figure 2).

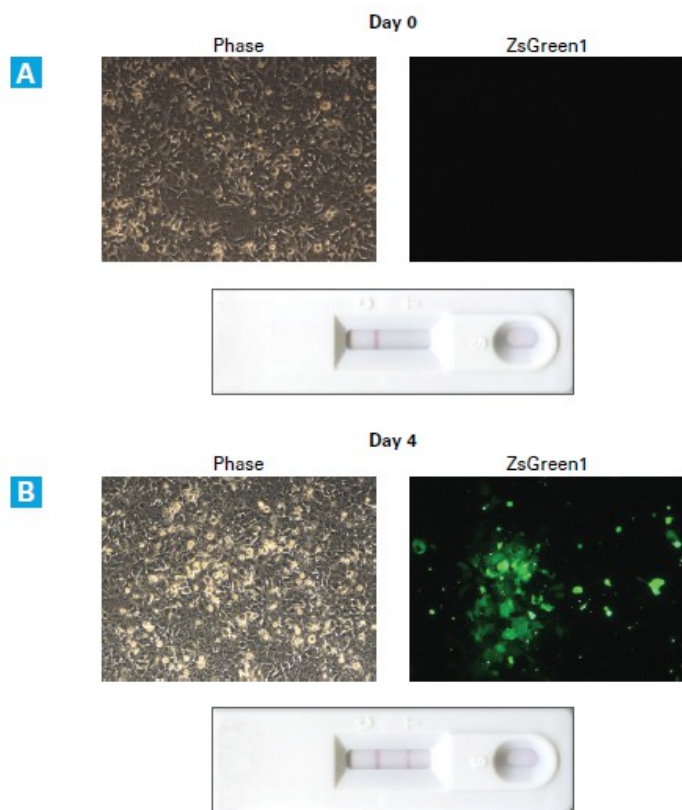


Figure 2. Early detection of adenoviral titers using Adeno-X GoStix. Viral titers were detected 4 days post-transfection with GoStix, which coincided with the early development of cytopathic effects and ZsGreen1 expression. **Panel A:** On Day 0, no viral titers were detected with GoStix. **Panel B:** On Day 4, viral titers were detected with GoStix, which coincided with appearance of cytopathic effects and fluorescence.

Rapid adenovirus detection during amplification

Adenovirus expressing ZsGreen1 was amplified in HEK293 cells 48 hr post-infection; and each day thereafter, the supernatant and the cell pellet were harvested and titers were quantified by counting fluorescent cells in an infectious unit (IFU) assay (Figure 3, Panel A). The increase in virus level in the cell pellets was mirrored by a proportional increase in the amount of virus collected from the culture supernatant. The amount of virus in the supernatant remained at <10% of the total virus output. The supernatant titer serves as a clear indicator of total yields. In addition to IFU determination, 20 μ l of each sample was applied to an Adeno-X GoStix cassette. The increase in band intensity paralleled the increase in infectious unit titer (Figure 3, Panel B).

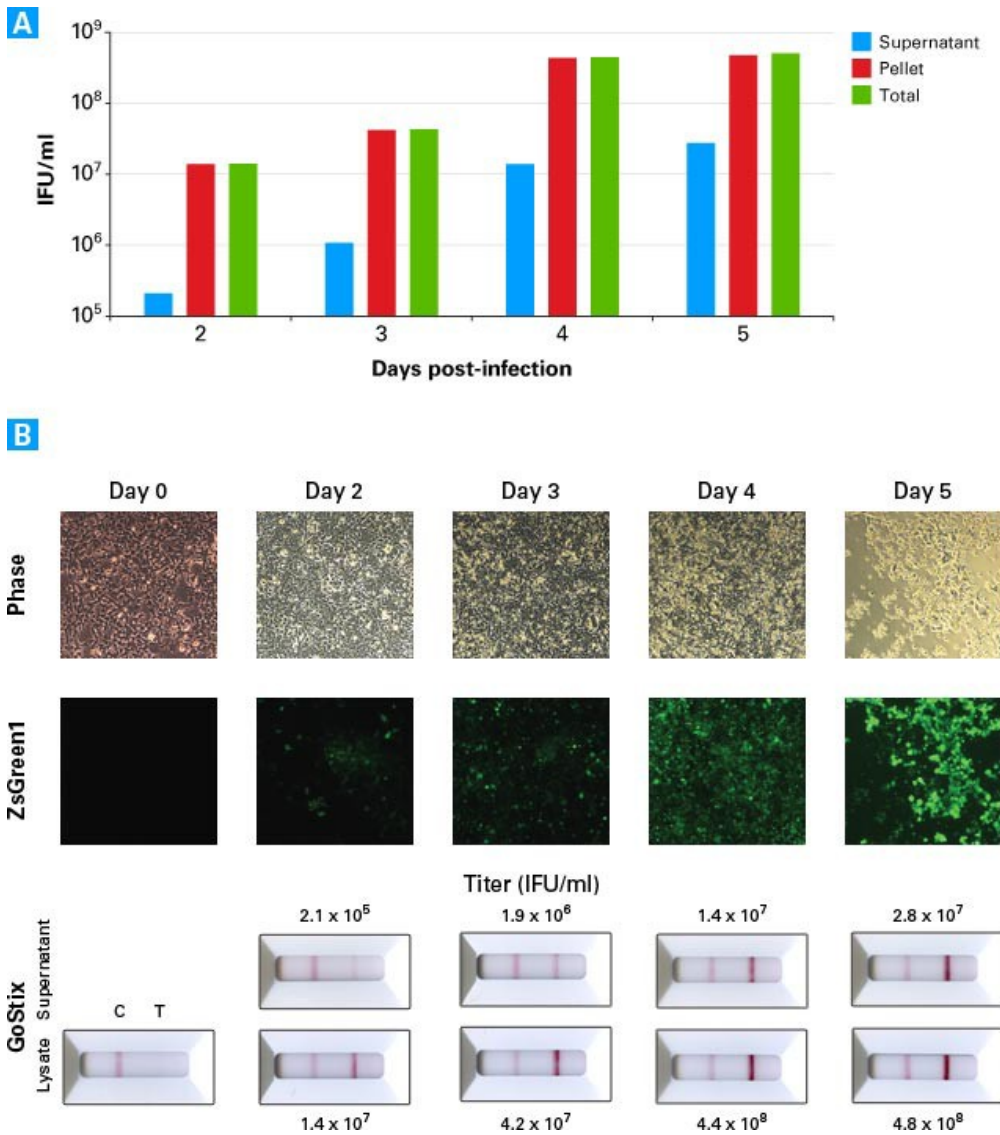


Figure 3. Rapid detection of adenoviral titer during amplification. Supernatant and pellet were collected each day post-infection to measure viral titer. As indicated by the graph, the supernatant titer serves as a clear indicator of total viral yields (**Panel A**). In addition to IFU determination, 20 µl of each sample was applied to an Adeno-X GoStix cassette. The increase in band intensity paralleled the increase in infectious unit titer (**Panel B**).

Conclusions

Adeno-X GoStix save time and effort during adenovirus production by providing a simple and rapid test for the status of adenovirus rescue even before cytopathic events are observed. Each test is sensitive enough to detect adenovirus in a small amount of crude supernatant, and is also used to ensure maximum yields during the adenovirus amplification process. The presence of a strong band in the cassette window ensures that high-titer adenovirus is harvested at the peak time.



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