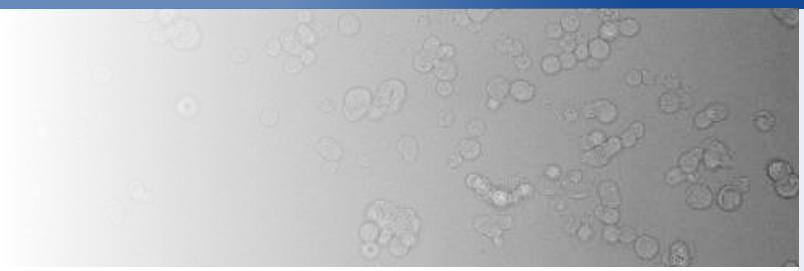
# Immediate Viral Inactivation Using Norgen's **Stool Nucleic Acid Preservative**

Application Note 96


Keywords

+ Virus
+ Adenovirus
+ DNA
+ RNA
+ Microbiota
+ Gut Microbiome
+ Transportation
+ Preservative
+ Stool
+ Storage
+ Method

## INTRODUCTION

Stool samples are valuable in the diagnosis of gastrointestinal conditions such as malabsorption, inflammatory diseases and digestive infections. Some stool tests can be easily done at the clinical point-of-care. However, for the accurate diagnosis of microbial infections (such as those attributed to parasites, viruses, bacteria or fungi), stool samples should be tested with molecular diagnostic methods such as RT-PCR and next generation sequencing (NGS). These tests are usually performed in centralized laboratories.

Traditional stool collection/preservation methods suffer from two issues: 1) higher costs incurred by cold-chain supplies and 2) higher risks of microbial infection during transportation. Typically, stool samples are collected in the morning to reach the laboratory and be processed on the same day. If stool samples need long-term transportation from remote locations to centralized laboratories, they should be subjected to refrigeration or cold chain transportation. In addition, stool samples are usually collected in Cary-Blair/Stuart transport medium, glycerol-phosphate buffer or alkaline peptone water. Such transportation media do not inactivate the infectious microbes and may infect transportation workers and point-of-care clinicians. Although some commercial products can fix stool samples using formalin, the formalin-based preservatives may impair the DNA/RNA or protein epitopes, thus hampering downstream applications such as PCR, NGS or immunoassays.

Norgen's proprietary stool sample collection and preservation products offer ease of use, long-term storage at room temperature, safe transportation and handling, and a wide selection of downstream applications. Stool samples can first be collected and preserved using Norgen's Stool Nucleic Acid Collection and Preservation Tubes (Cat. 45630, 45660) and Stool Nucle-

***Device Quick Links***

**Click to find out more on Norgen's website.**

Stool Nucleic Acid Collection and Preservation Tubes

Stool Nucleic Acid Collection and Preservation System

Stool DNA Isolation Kit

Stool Nucleic Acid Isolation Kit

ic Acid Collection and Preservation System (Cat. 63700). The DNA and RNA can then be purified with the Stool DNA Isolation Kit (Cat. 27600, 65600) or Stool Nucleic Acid Isolation Kit (Cat. 45600). The purified DNA/RNA samples can be utilized in PCR and 16S NGS. In this study, we aim to evaluate the ability of adenoviral inactivation using Norgen's Stool Nucleic Acid Preservative.

## MATERIALS AND METHODS

**Inactivation of Adenovirus Viral Particles**

Human embryonic kidney 293A (HEK293A) cell line (Graham et al, 1977; ATCC CRL-1573) was used in this experiment. Cells were cultured as monolayers in EMEM (Wisent Inc.) supplemented with 1% (v/v) Penicillin/Streptomycin (Wisent Inc.) and 10% (v/v) heat-inactivated FBS (Gibco Inc.) at 37°C with 5% $CO_2$.

A recombinant adenovirus type 5 carrying a GFP transgene cassette (Ad-GFP) was used. Infection of HEK293A cells was carried out in 48-well plates ($5x10^4$ cells/well) by using Ad-GFP with a titer of $5x10^4$ IVP/µL (infectious viral particles/µL), at 0.5 MOI (multiplicity of infection). For each well of a 48-well plate, 5 µL of Ad-GFP was mixed in 995 µL of PBS++ (in a total volume of 1 mL) and, out of this 1 mL mixture, 100 µL was added to each well. The 48-well plates were then incubated for 1 hour at 37°C with 5% $CO_2$, with gentle swirling of the plates every 15 minutes. Thereafter, 0.5 mL of the culture medium was added to each well and the plates were incubated for 24 hours.

To evaluate the effect of Norgen's Stool Nucleic Acid Preservative on adenoviral inactivation, 5 µL of Stool Nucleic Acid Preservative and 10 uL of 1 mg/mL Propidium Iodide (PI, final concentration 10 µg/mL, to visualize dead cells) were dissolved in 985 µL of PBS++ (1:200, v/v) and 100 µL of this mixture was added to one well after aspirating the medium. As a negative control, 100 µL of PBS++ containing PI (10 µL of 1 mg/mL PI in 990 µL of PBS++) was added to a separate well after removing the medium. The brightfield and fluorescent images were immediately acquired using a ZOE Fluorescent Cell Imager (Bio-Rad) with an excitation wavelength of 480/17 nm and emission wavelength of 517/23 nm for GFP signal (green), and an excitation wavelength of 556/20 nm and emission wavelength of 615/61 nm for PI or the dead cell signal (red).

## RESULTS AND DISCUSSION

The PBS++ treated cells (control) showed clear green fluorescence as opposed to the stool preservative treated cells (test) that did not fluoresce (**Figure 1a**). This implies that the PBS++ treatment neither killed the cells nor quenched the GFP signal, as opposed to the stool preservative which inactivated the adenovirus. Moreover, the PI stain appeared in the stool preservative treated cells (test) but not in the PBS++ treated cells (control) (**Figure 1b**). This result indicates that the stool preservative killed the HEK293A cells (test) and prevented the adenovirus from infecting them.

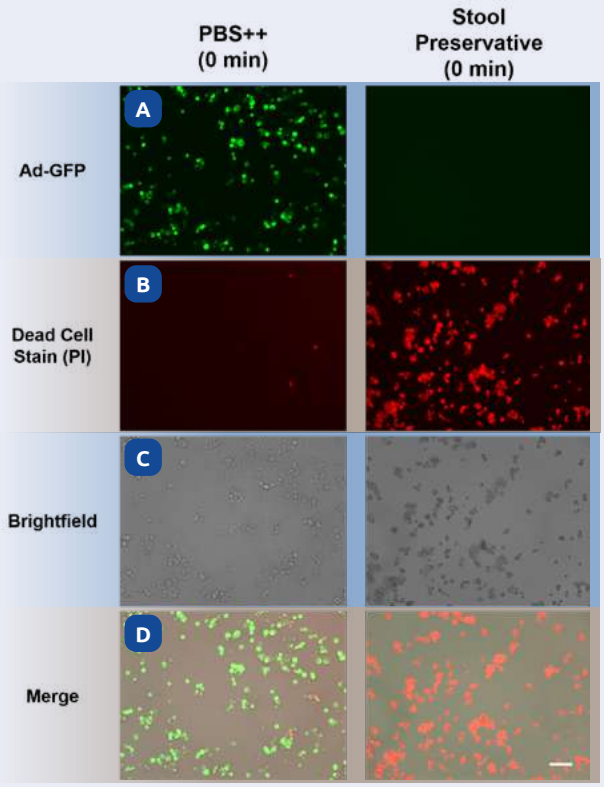



**Figure 1. Images of Ad-GFP-infected HEK293A cells acquired using fluorescent microscopy.** Monolayer HEK293A cells were infected with Ad-GFP. Twenty-four hours after infection, cells were stained with propidium iodide (PI), a red fluorescent dye which specifically stains the dead cells. **Left:** After treating the cells with PBS++ at 0 minutes, Ad-GFP-infected HEK293A cells displayed a strong GFP signal (green fluorescence) and few dead cells (red fluorescence) can be observed. **Right:** After treating the cells with Norgen's Stool Nucleic Acid Preservative, the GFP signal disappeared and all cells were dead, suggesting a complete inactivation of Ad-GFP. Scale bar: 100 µm.

## CONCLUSIONS

Norgen's Stool Nucleic Acid Preservative has been formulated to inactivate enveloped RNA viruses (such as SARS-CoV-2 and HIV) and more resilient DNA viruses with capsid proteins (such as adenoviruses). The present study demonstrates the immediate viral inactivation capacity of Norgen's Stool Nucleic Acid Preservative. Overall, the use of stool samples collected in Norgen's Stool Nucleic Acid Preservative allows long-term storage at room temperature, a wide variety of downstream applications such as PCR and NGS, and safe handling of otherwise potentially infectious samples during sample collection, storage, transportation and molecular testing.

| Related Products | Research Use | CE Marked |
|---|---|---|
| Stool Nucleic Acid Collection and Preservation Tubes | 45630, 45660 | Dx45660 |
| Stool Nucleic Acid Collection and Preservation System | 63700 | - |
| Stool DNA Isolation Kit | 27600, 65600 | - |
| Stool Nucleic Acid Isolation Kit | 45600 | - |

**Norgen Biotek Corp.**
3430 Schmon Parkway,
Thorold, ON, Canada, L2V 4Y6

**E** info@norgenbiotek.com
**T** 1 866 NORGENB
**www.norgenbiotek.com**