

Comparison of Exgene™ Viral DNA/RNA kit to other commercial kits for pathogen nucleic acid extraction from rooster stool

Experimental Conditions

Materials Required

- Exgene™ Viral DNA/RNA (128-150)
- Buffer FL, 70 ml (115-910)
- 2 ml Glass Bead Tube (406-893)
- 1.5 ml microcentrifuge tube
- Microcentrifuge ($\leq 14,000 \times g$)
- Vortex mixer
- Pipette & sterilized pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice

Sample Information

Pathogen	<i>Mycoplasma Gallisepticum</i> (MG)	Infectious Bronchitis Virus (IBV)
Target	Bacterial DNA	Viral RNA
Sample	Pathogen-infected rooster stool	
Sample amount	200 mg	
Elution volume	50 μ l	

Protocol

Exgene™ Viral DNA/RNA Protocol

* For more details and methods, please refer to [the handbook of Exgene™ Viral DNA/RNA](#).

Preparation of Proteinase K and Carrier RNA Solution

• Proteinase K solution

To obtain a 20 mg/ml Proteinase K solution, add 650 μ l of PK Storage Buffer to the tube of lyophilized 13 mg of Proteinase K, and mix carefully to avoid foaming.

• Carrier RNA solution

To obtain a 1 μ g/ μ l Carrier RNA solution, add 370 μ l of Nuclease-free water to the tube containing lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots.

Sample Preparation

• Pathogen-infected rooster stool

1. Add 200 mg of each rooster stool infected with pathogen to each 2 ml Glass Bead Tube.
2. Add 1 ml of Buffer FL and 25 μ l of Proteinase K solution (20 mg/ml) to the tube. Vortex for 1 min or until the stool samples is thoroughly homogenized.
3. Incubate for 5 min at 65 °C.
4. Centrifuge at 10,000 $\times g$ for 10 min at room temperature.
5. Transfer 200 μ l of supernatant to the each 1.5 ml microcentrifuge tube.
6. The subsequent protocol follows **step 3 on page 10 of protocol in the Exgene™ Viral DNA/RNA handbook**.

Result

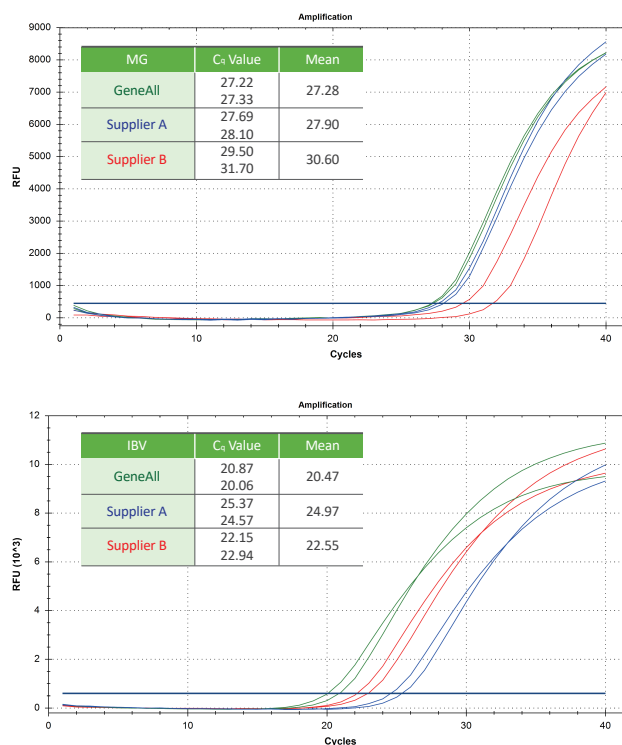


Figure 1. Comparison of bacterial DNA and viral RNA detection by qPCR analysis of rooster stool samples obtained from Exgene™ Viral DNA/RNA kit and competitor kits. Nucleic acids were extracted from stool samples of two types of pathogen-infected roosters using GeneAll's Exgene™ Viral DNA/RNA kit ('Green') and competitor kits ('Blue' and 'Red'), with each extraction performed in duplicate.

Real-time PCR was performed on following extraction of bacterial DNA and viral RNA. Each pathogen nucleic acid was amplified with target-specific primers and probes. The PCR data shows that Exgene™ Viral DNA/RNA kit is more efficient in extracting and detecting the nucleic acids of interest from the rooster stool samples.

- Real-time PCR system: CFX96™ System (1855201, supplier B)
- qPCR kit: RealAmp™ 2X qPCR Master Mix (801-020)
- RT-qPCR kit: HyperScript™ One-step RT-PCR Master Mix (602-110)