Performance comparison of Exgene™ Viral DNA/RNA and competitors' kits from buccal swab of pathogen-infected rooster

Experimental Conditions

Materials Required

- Exgene™ Viral DNA/RNA (128-150)
- Sterilized cotton swab for sample collection
- 1X PBS (Phosphate-buffered saline), pH 7.4 (SM-P04-100)
- 1.5 ml microcentrifuge tube
- Microcentrifuge (≤14,000 x g)
- Vortex mixer
- Pipette & sterilized pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice

Sample Information

Pathogen	Mycoplasma Gallisepticum (MG)	Infectious Bronchitis Virus (IBV)
Target	Bacterial DNA	Viral RNA
Sample	Buccal swab of pathogen-infected rooster	
Sample amount	200 μΙ	
Elution volume	50 μΙ	

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

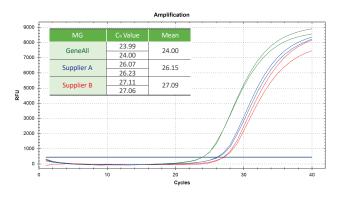
To obtain a 1 $\mu g/\mu l$ Carrier RNA solution, add 370 μl of Nuclease-free water to the tube containing lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots.

Sample Preparation

· Pathogen-infected rooster swab

- 1. Collect the buccal epithelial cell by rubbing the inside of the cheek of each pathogen-infected rooster with cotton swab.
- Place the swab in each 1.5 ml microcentrifuge tube (not provided). Clip off handle of brush with sterile sharp blade or wire cutter.
- 3. Add $400 \sim 500 \,\mu l$ of 1X PBS to the tube. Vortex for 1 min.
- 4. Pipet 10 μ l of Proteinase K solution (20 mg/ml) into the bottom of a new 1.5 ml microcentrifuge tube (not provided).
- 5. Transfer 200 μl of each sample to the new 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



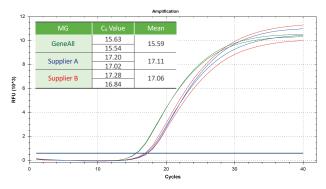


Figure 1. Results of real-time PCR using viral/pathogen DNA/RNA extraction kits.

Nucleic acids were extracted from pathogen-infected rooster's oral epithelial cells using Exgene™ Viral DNA/RNA kit (Green) and other two competitors' equivalent kits (Blue & Red) in duplicate. Real-time PCR was performed with extracted DNA/RNA, as template, to assess the performance.

- 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)