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
- 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 µl	
Elution volume	50 µl	

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 µl 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 µg/µl 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.
  2. 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 400~500 µl of 1X PBS to the tube. Vortex for 1 min.
  4. Pipet 10 µl 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

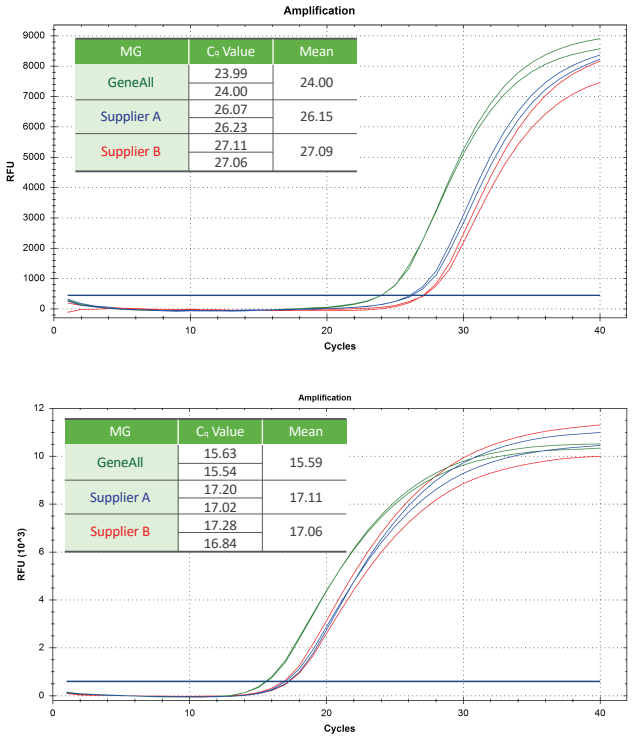

Figure 1. Results of real-time PCR using viral/pathogen DNA/RNA extraction kits.
Nucleic acids were extracted from pathogen-infected rooster's oral epithelial cells using Exgene™ Viral DNA/RNA kit (Green) and other two competitors' equivalent kits (Blue & Red) in duplicate. Real-time PCR was performed 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)