

# Comparison of the Pathogen Purification Performance of Exgene™ Viral DNA/RNA to Other Commercial Kits

## Experimental Conditions

### Materials Required

- Exgene™ Viral DNA/RNA (128-150)
- 5 ml conical tube (for sample preparation)
- Vortex mixer
- Microcentrifuge ( $\leq 15,000 \times g$ )
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)
- Ice (for maintenance normal state and freeze-thaw of Carrier RNA solution)

### Sample Information

- Sample type: cultured virus and bacteria
  - Infectious bronchitis virus (IBV,  $10^{3.5} \text{EID}_{50}$ )
  - Rabies virus (RV,  $10^{3.5} \text{LD}_{50}$ )
  - Japanese encephalitis virus (JEV,  $10^{5.0} \text{TICD}_{50}$ )
  - *Mycoplasma gallisepticum* (MG,  $1 \times 10^5 \text{CCU}$ )
- Extraction conditions
  - Sample amount: 200  $\mu\text{l}$
  - Elution volume: 100  $\mu\text{l}$
  - Extraction protocol: Viral\_Normal (operation time: 29' 35")

### Sample Preparation

1. Mix the all cultured viruses and bacteria medium to 5 ml conical tube and extract the 200  $\mu\text{l}$  samples from the mixture.
2. One sample is according to Exgene™ Viral DNA/RNA protocol, the other samples are according to manual method of viral DNA/RNA extraction kits each from two different suppliers for comparison.

## Protocol

### Exgene™ Viral DNA/RNA Extraction Kit Protocol

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

1. Add 10  $\mu\text{l}$  of Proteinase K solution (20 mg/ml) to 1.5 ml microcentrifuge tube.
2. Transfer the 200  $\mu\text{l}$  of mixed samples and add 200  $\mu\text{l}$  of Buffer BL to the tube.
3. Add 7  $\mu\text{l}$  of Carrier RNA solution (1  $\mu\text{g}/\mu\text{l}$ ) to the tube and mix thoroughly by vortexing for 10 s.
4. Incubate the tube at 56 °C for 10 min and spin down briefly to remove any drops.
5. Add 400  $\mu\text{l}$  of Buffer RB1 to the tube and vortex for 10 s.
6. Transfer the mixture to a Column Type S and centrifuge at  $\geq 10,000 \times g$  for 1 min at room temperature. Discard the pass-through and reinsert.
7. Add 500  $\mu\text{l}$  of Buffer BW to the column and centrifuge at  $\geq 10,000 \times g$  for 1 min at room temperature. Discard the pass-through and reinsert.
8. Add 700  $\mu\text{l}$  of Buffer TW to the column and centrifuge at  $\geq 10,000 \times g$  for 1 min at room temperature. Discard the pass-through and reinsert.
9. Centrifuge at full speed for 1 min at room temperature and transfer the column to a new 1.5 ml microcentrifuge tube.
10. Add 20–50  $\mu\text{l}$  of Nuclease-free water to the center of the membrane in the column. Stand for 1 min and centrifuge at  $\geq 10,000 \times g$  for 1 min at room temperature.

## Result

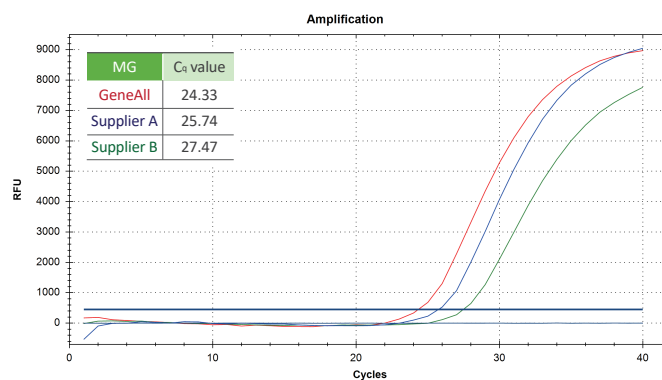
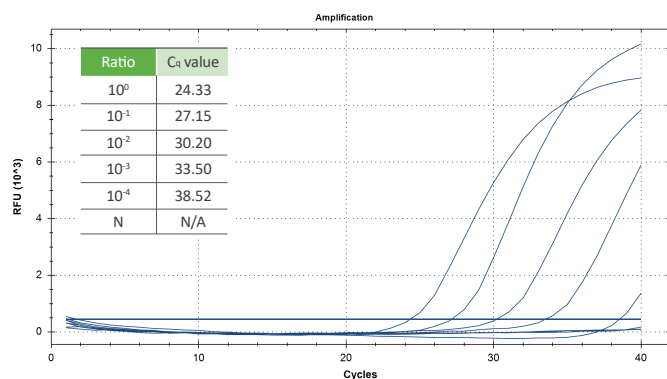


Figure 1. Comparison of  $C_t$  value of DNA template extracted from the *Mycoplasma gallisepticum* (MG).

The DNA templates were extracted from the *Mycoplasma gallisepticum* (MG) using Exgene™ Viral DNA/RNA (red line) and viral DNA/RNA extraction kits (manual method) each from two different suppliers (blue and green line). Eluted DNA templates were analyzed with a TaqMan-based real-time PCR assay using CFX-96.

- Red line: GeneAll® Exgene™ Viral DNA/RNA
- Blue line: viral DNA/RNA extraction kit (supplier A)
- Green line: viral DNA/RNA extraction kit (supplier B)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391A)
- Target gene: none specific

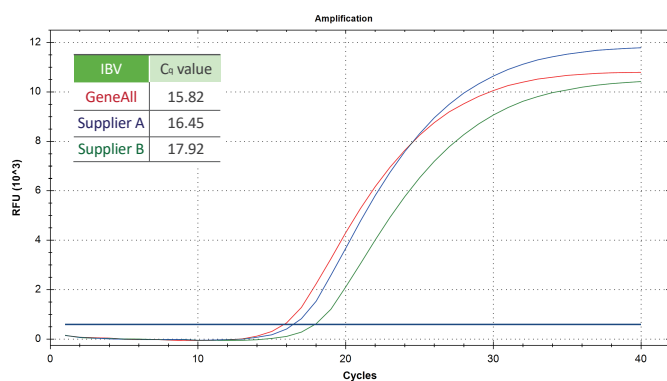
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**Figure 2. Analysis of extraction sensitivity on serial dilutions of *Mycoplasma gallisepticum* (MG).**

The DNA templates were extracted from a 10-fold serial dilution of *Mycoplasma gallisepticum* (MG) using Exgene™ Viral DNA/RNA. All eluates were analyzed with a TaqMan-based real-time PCR assay using CFX-96.

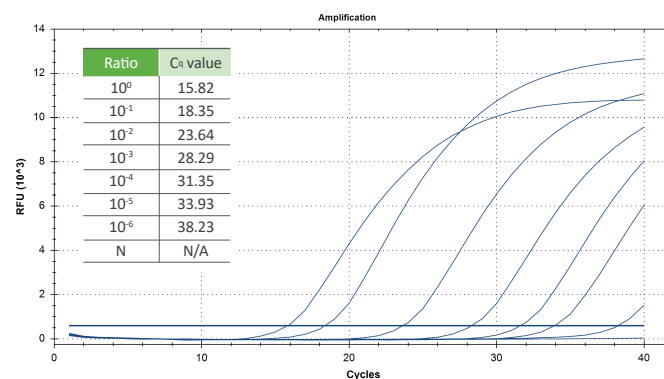
- N: negative control (nuclease-free water)
- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391AT)
- Target gene: none specific



**Figure 3. Comparison of C<sub>t</sub> value of DNA template extracted from the Infectious bronchitis virus (IBV).**

The RNA templates were extracted from the Infectious bronchitis virus (IBV) using Exgene™ Viral DNA/RNA (red line) and viral DNA/RNA extraction kits (manual method) each from two different suppliers (blue and green line). Eluted RNA templates were synthesized to cDNA with reverse transcription; and then analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96.

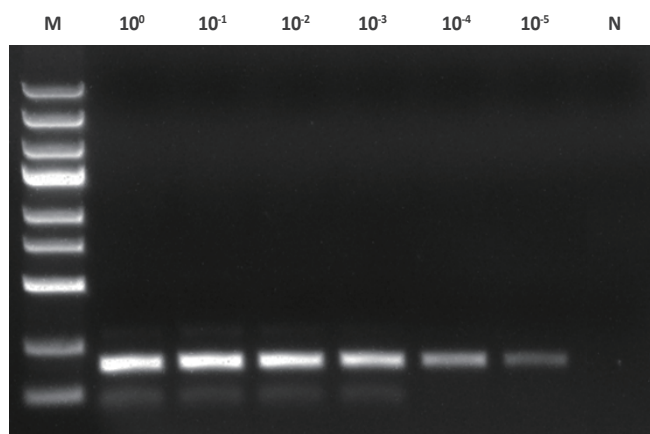
- Red line: GeneAll® Exgene™ Viral DNA/RNA
- Blue line: viral DNA/RNA extraction kit (supplier A)
- Green line: viral DNA/RNA extraction kit (supplier B)
- Real-time PCR system: CFX-96™ System (1855201)
- RT-qPCR kit: GeneAll® HyperScript™ 2X One-step RT-PCR Master Mix (602-110)
- Target gene: none specific



**Figure 4. Analysis of extraction sensitivity on serial dilutions of Infectious bronchitis virus (IBV).**

The RNA template were extracted from a 10-fold serial dilution of Infectious bronchitis virus (IBV) using Exgene™ Viral DNA/RNA. All elutes were analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96.

- N: negative control (nuclease-free water)
- Real-time PCR system: CFX-96™ System (1855201)
- RT-qPCR kit: GeneAll® HyperScript™ 2X One-step RT-PCR Master Mix (602-110)
- Target gene: none specific

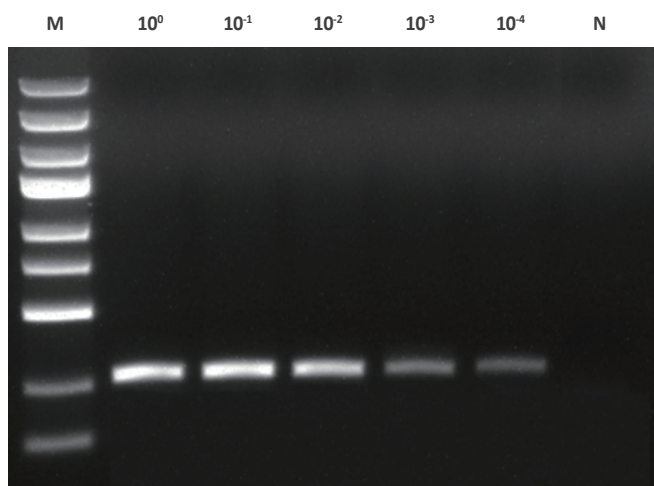


**Figure 5. Analysis of extraction sensitivity on dilutions of Rabies virus (RV).**

The RNA template were extracted from a 10-fold serial dilution from 10<sup>0</sup> to 10<sup>-5</sup> of known positive Rabies virus (RV) samples using Exgene™ Viral DNA/RNA. All eluates were analyzed using conventional reverse transcription PCR (one-step RT-PCR) assay.

- M: GeneAll® GENESTA™ 250 bp DNA ladder (GA-025)
- N: negative control (nuclease-free water)
- Target gene (PCR product size): Jecom (100 bp)
- PCR system: MultiGene™ Optimax thermal cycler (TC9610, supplier L)
- RT-PCR kit: GeneAll® HyperScript™ One-Step RT-PCR Master Mix, 0.5 ml x 2 (602-125)
- Electrophoresis conditions: 1.2% agarose, 110 V, 30 min, 10 µl

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**Figure 6. Analysis of extraction sensitivity on dilutions of Japanese encephalitis virus (JEV).**  
The RNA template were extracted from a 10-fold serial dilution from  $10^0$  to  $10^{-4}$  of known positive Japanese encephalitis virus (JEV) samples using Exgene™ Viral DNA/RNA. All eluates were analyzed using conventional reverse transcription PCR (one-step RT-PCR) assay.

- M: GENESTA™ 250 bp DNA ladder (GA-025)
- N: negative control (nuclease-free water)
- Target gene (PCR product size): omRABV (192 bp)
- PCR system: MultiGene™ Optimax thermal cycler (TC9610, supplier L)
- RT-PCR kit: GeneAll® HyperScript™ 2X One-step RT-PCR Master Mix (602-110)
- Electrophoresis conditions: 1.2% agarose, 110V, 30 min, 10  $\mu$ l