Efficient purification of high quality viral nucleic acids from a wide range of sample materials using GENTi™ Advanced Viral DNA/RNA Extraction Kit

Experimental Conditions

Materials Required

- GENTi™ 32 Advanced Automatic Extraction System (GTI032A)
- GENTi™ Advanced Viral DNA/RNA Extraction Kit (902-096A)
- 5 ml conical screw tube (for sample preparation)
- Pipette & sterile pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

- Sample type : Cultured virus and bacteria
  - Infectious bronchitis virus (IBV, $10^{3.5}EID_{50}$)
  - Rabies virus (RV, $10^{3.5}LD_{50}$)
  - Japanese encephalitis virus (JEV, $10^{5.0}TICD_{50}$)
  - *Mycoplasma gallisepticum* (MG, 1 x 10^5CCU)

- Extraction conditions
  - Sample amount : 200 µl
  - Elution volume : 100 µl
  - Extraction protocol : Viral_Normal (operation time : 29’ 35”)

Sample Preparation

1. Mix the all cultured virus and bacteria medium to 5 ml conical tube and extract the 200 µl sample from the mixture.
2. One sample is according to **GENTi™ Advanced Viral DNA/RNA Extraction Kit protocol**, the other sample is according to manual method of viral DNA/RNA extraction kit each from two different suppliers for comparison.

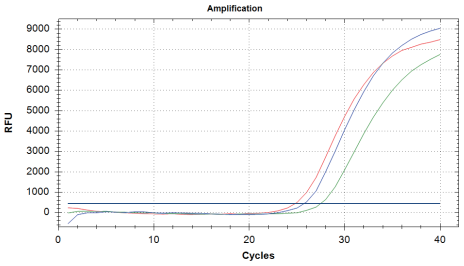
Protocol

GENTi™ Advanced Viral DNA/RNA Extraction Kit protocol

*** For more details and methods, please refer to the handbook of GENTi™ Advanced Viral DNA/RNA Extraction Kit.**

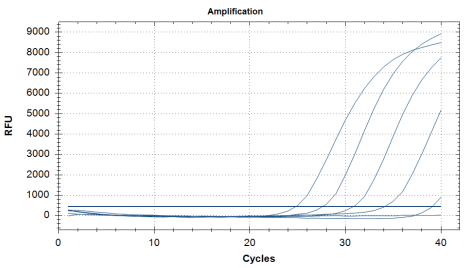
1. Add 7 µl of dissolved Carrier RNA (1 µg/µl) to 1st/7th well of plate type cartridge.
2. Add 200 µl of samples to 1st/7th well.
3. Load the plate type cartridge on the tray of GENTi™ 32 Advanced Automatic Extraction System.
4. Insert the magnetic rod cover to the end to strip bracket.
5. Select the correct extraction protocol and operate the extraction system.

Result



MG	Cq value
GeneAll	24.81
Supplier A	25.74
Supplier B	27.47

Figure 1. Comparison of Cq value of DNA template extracted from the *Mycoplasma gallisepticum* (MG).
The DNA template was extracted from the *Mycoplasma gallisepticum* (MG) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System (red line) and viral DNA/RNA extraction kit (manual method) each from two different suppliers (blue and green line). Eluted DNA template was analyzed with a TaqMan-based real-time PCR assay using CFX-96.
Red line : GENTi™ Advanced Viral DNA/RNA Extraction Kit
Blue line : Supplier A manual extraction kit
Green line : Supplier B manual extraction kit
- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
- Target gene : None specific



Ratio	Cq value
10^0	24.81
10^{-1}	27.61
10^{-2}	30.84
10^{-3}	34.81
10^{-4}	39.09
N	N/A

Figure 2. Analysis of extraction sensitivity on serial dilutions of *Mycoplasma gallisepticum* (MG).
The DNA template was extracted from a 10-fold serial dilution of *Mycoplasma gallisepticum* (MG) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System. All eluates were analyzed with a TaqMan-based real-time PCR assay using CFX-96.
- N : Negative control (Nuclease-free water)
- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
- Target gene : None specific

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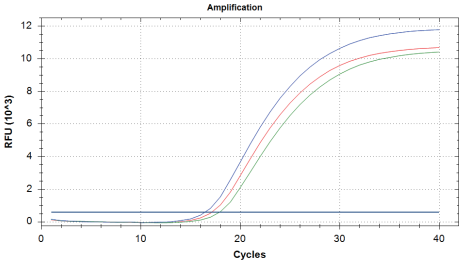
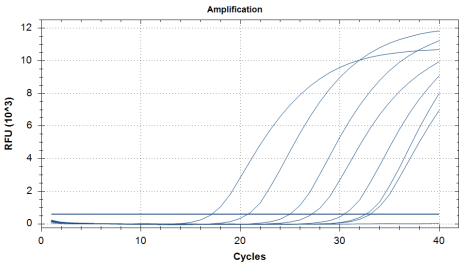


MG	C_q value
GeneAll	17.14
Supplier A	16.45
Supplier B	17.92

Figure 3. Comparison of C_q value of RNA template extracted from the Infectious bronchitis virus (IBV).
The RNA template was extracted from the Infectious bronchitis virus (IBV) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System (red line) and viral DNA/RNA extraction kit (manual method) each from two different suppliers (blue and green line). Eluted RNA template was synthesized to cDNA with reverse transcription; and then analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96.
Red line : GENTi™ Advanced Viral DNA/RNA Extraction Kit
Blue line : Supplier A manual extraction kit
Green line : Supplier B manual extraction kit
- PCR instrument : CFX-96 (1855201, Supplier : B)
- RT-qPCR kit : 2X 1 Step RT-qPCR Master Mix [for probe] (QRT1-XV-100R)
- Target gene : None specific



Ratio	C_q value
10^0	17.14
10^{-1}	20.76
10^{-2}	25.01
10^{-3}	27.19
10^{-4}	30.44
10^{-5}	32.61
10^{-6}	33.09
N	N/A

Figure 4. Analysis of extraction sensitivity on serial dilutions of Infectious bronchitis virus (IBV).
The RNA template was extracted from a 10-fold serial dilution of Infectious bronchitis virus (IBV) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System. All elutes were analyzed with TaqMan-based One-Step RT-qPCR assay using CFX-96.
- N : Negative control (Nuclease-free water)
- PCR instrument : CFX-96 (1855201)
- RT-qPCR kit : 2X 1 Step RT-qPCR Master Mix [for probe] (QRT1-XV-100R)
- Target gene : None specific

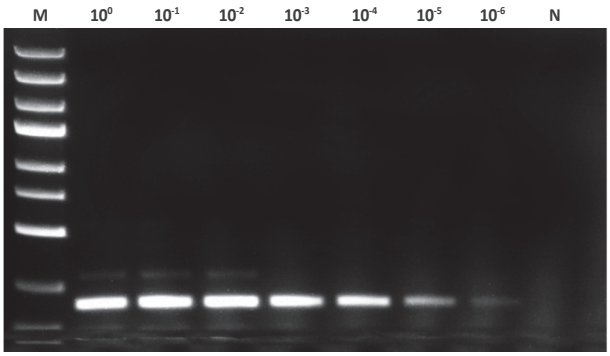


Figure 5. Analysis of extraction sensitivity on dilutions of Rabies virus (RV).
The RNA template was extracted from a 10-fold serial dilution of known positive Rabies virus (RV) samples using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System. All eluates were analyzed using conventional reverse transcription PCR (RT-PCR) assay.
- N : Negative control (Nuclease-free water)
- Target gene (PCR product size) : Jecom (100 bp)
- PCR instrument : MultiGene™ Optimax thermal cycler (TC9610)
- RT-PCR kit : 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

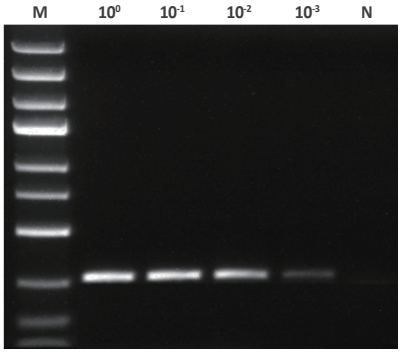


Figure 6. Analysis of extraction sensitivity on dilutions of Japanese encephalitis virus (JEV).
The RNA template was extracted from a 10-fold serial dilution of known positive Japanese encephalitis virus (JEV) samples using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System. All eluates were analyzed using conventional reverse transcription PCR (RT-PCR) assay.
- N : Negative control (Nuclease-free water)
- Target gene (PCR product size) : omRABV (192 bp)
- PCR instrument : MultiGene™ Optimax thermal cycler (TC9610)
- RT-PCR kit : 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