

High-quality Nucleic Acids from Pathogen-infected Stool Samples with 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 (902-048A/902-096A)
- ♦ Stainless bead, 6 mm
- ♦ 1X PBS (Phosphate-buffered saline), pH 7.4
- ♦ 5 ml conical tube
- ♦ Pipette & sterile pipette tips
- ♦ Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

- ♦ Sample type: stool infected with pathogen
 - Viral RNA: Infectious bronchitis virus (IBV)
 - Bacterial DNA: *Mycoplasma gallisepticum* (MG)
- ♦ Extraction conditions
 - Sample amount: 200 µl
 - Elution volume: 100 µl
 - Extraction protocol: Viral_Normal (operation time: 29' 35")

Sample Preparation

1. Prepare each 200 mg stool infected with IBV or MG and transfer the samples to each 5 ml conical tube with 1X PBS and 6 mm stainless bead.
2. Vortex for 1 min or until the stool sample is thoroughly homogenized and incubate for 1 min at room temperature.
3. The next step is according to **GENTi™ Advanced Viral DNA/RNA Extraction Kit protocol**.

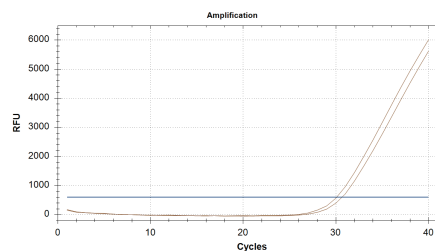
Protocol

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

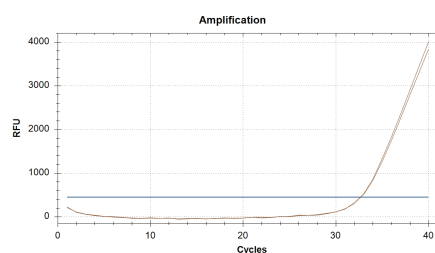


MG	C _t value
#1	32.60
#2	32.69

Figure 1. Result of C_t value of DNA template extracted from infected stool.

The DNA templates were extracted from the stool infected with *Mycoplasma gallisepticum* (MG) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System. Eluted DNA templates were analyzed with a TaqMan-based real-time PCR assay using CFX-96. Each eluate was analyzed in duplicate.

- Real-time PCR system: CFX-96™ System (1855201)
- qPCR kit: Probe qPCR Mix (RR391A)
- Target gene: none specific



IBV	C _t value
#1	30.08
#2	30.65

Figure 2. Result of C_t value of RNA template extracted from infected stool.

The RNA templates were extracted from the stool infected with Infectious bronchitis virus (IBV) using GENTi™ Advanced Viral DNA/RNA Extraction Kit on GENTi™ 32 Advanced Automatic Extraction System. Eluted RNA templates were synthesized to cDNA with reverse transcription; and then analyzed with TaqMan-based one-step RT-qPCR assay using CFX-96. Each eluate was analyzed in duplicate.

- 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