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 (902-096A)
- Stainless bead, 6 mm (SUS 304, Supplier : S)
- 1X PBS (Phosphate-buffered saline)
- 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**.

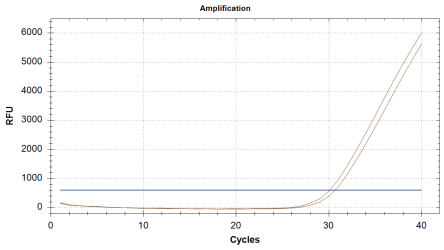
Protocol

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.**

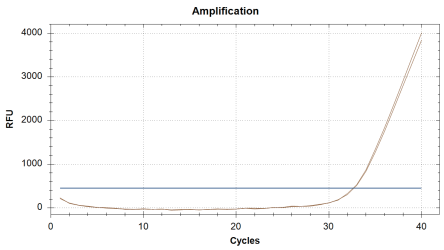
1. Add 7 µl of dissolved Carrier RNA (1 µg/µl) to 1st/7th well of plate type cartridge.
2. Add 200 µl of samples to 1st/7th well.
3. Load the plate type cartridge on the tray of GENTi™ 32 Advanced Automatic Extraction System.
4. Insert the magnetic rod cover to the end to strip bracket.
5. Select the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

Result



MG	C_q value
#1	32.60
#2	32.69

Figure 1. Result of C_q 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.
- PCR instrument : CFX-96 (1855201)
- qPCR kit : Probe qPCR Mix (RR391A)
- Target gene : None specific



IBV	C_q value
#1	30.08
#2	30.65

Figure 2. Result of C_q 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.
- PCR instrument : CFX-96 (1855201)
- RT-qPCR kit : 2X 1 Step RT-qPCR Master Mix (for probe) (QRT1-XV-100R)
- Target gene : None specific