Comparison data of Ribospin™ Pathogen/TNA with other commercial kits from pathogen-infected rooster whole blood

Experimental Conditions

Materials Required

- Ribospin™ Pathogen/TNA (341-150)
- Commercial kit (supplier A & B)
- Syringe for animal whole blood collection
- 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.)

Sample Information

Pathogen	*Mycoplasma Gallisepticum* (MG)	Infectious Bronchitis Virus (IBV)
Target	Pathogen DNA/RNA	
Sample	Pathogen-infected rooster whole blood	
Sample amount	200 µl	
Elution volume	50 µl	

Protocol

Ribospin™ Pathogen/TNA Protocol

* **For more details and methods, please refer to the handbook of Ribospin™ Pathogen/TNA.**

Preparation of Proteinase K solution

- **Proteinase K solution**
  Before start experiment, Proteinase K (24 mg) mix to 1.2 ml of PK Storage Buffer carefully to avoid foaming.

Protocol for Pathogen-infected rooster whole blood

1. Transfer 200 µl of each pathogen-infected rooster whole blood sample to the 1.5 ml microcentrifuge tube.
2. Add 200 µl of Buffer SL to the sample and vortex to mix thoroughly.
3. Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer BL to the sample. Vortex vigorously to mix thoroughly.
4. Incubate at RT for 10 min.
5. Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.
6. Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.
7. Repeat step 6 with the remainder of the sample.
8. Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.
9. Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.
10. Centrifuge at full speed for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.
11. Add 50 µl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.
12. Centrifuge at full speed for 1 min.

Result

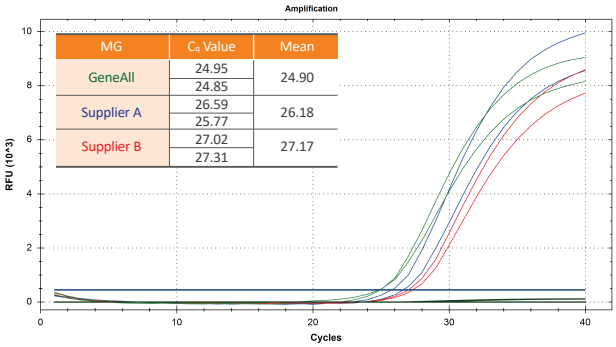

Figure 1. The results of qPCR for *Mycoplasma Gallisepticum* (MG)
Ribospin™ Pathogen/TNA(Green) and two equivalent kits from competitors (Blue & Red) were used in duplicate to extract TNA from whole blood of MG-infected rooster. qPCR was performed with extracted TNA as a template to assess the performance.
• Real-time PCR system: CFX96™ System (1855201, supplier B)
• qPCR kit: RealAmp™ 2X qPCR Master Mix (801-020)

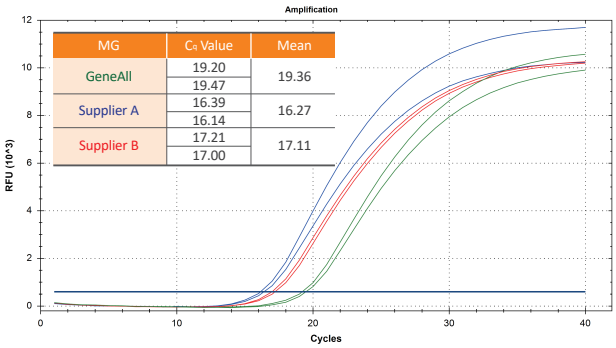

Figure 2. The results of qPCR for Infectious Bronchitis Virus (IBV)
Ribospin™ Pathogen/TNA(Green) and other two equivalent kits from competitors (Blue & Red) were used in duplicate to extract TNA from whole blood of IBV-infected rooster. qPCR was performed with extracted TNA as a template to assess the performance.
• Real-time PCR system: CFX96™ System (1855201, supplier B)
• RT-qPCR kit: HyperScript™ One-step RT-PCR Master Mix (602-110)