

# 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 ( $\leq 14,000 \times g$ )
- Vortex mixer
- Pipette & sterilized pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

### Sample Information

| Pathogen       | <i>Mycoplasma Gallisepticum</i> (MG)  | Infectious Bronchitis Virus (IBV) |
|----------------|---------------------------------------|-----------------------------------|
| Target         | Pathogen DNA/RNA                      |                                   |
| Sample         | Pathogen-infected rooster whole blood |                                   |
| Sample amount  | 200 $\mu$ l                           |                                   |
| Elution volume | 50 $\mu$ l                            |                                   |

## 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

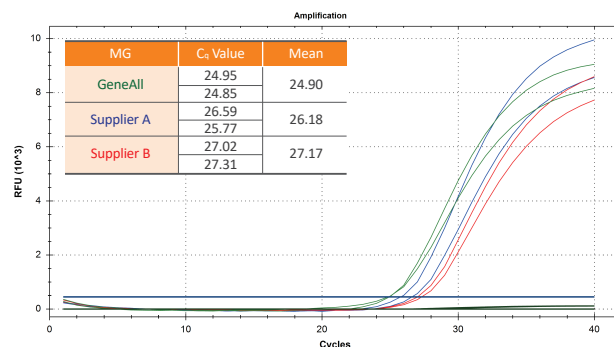
Before start experiment, Proteinase K (24 mg) mix to 1.2 ml of PK Storage Buffer carefully to avoid foaming.

### Protocol for Pathogen-infected rooster whole blood

1. Transfer 200  $\mu$ l of each pathogen-infected rooster whole blood sample to the 1.5 ml microcentrifuge tube.
2. Add 200  $\mu$ l of Buffer SL to the sample and vortex to mix thoroughly.
3. Add 20  $\mu$ l of Proteinase K solution (20 mg/ml, provided) and 200  $\mu$ l of Buffer BL to the sample. Vortex vigorously to mix thoroughly.
4. Incubate at RT for 10 min.
5. Add 300  $\mu$ l 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  $10,000 \times g$  above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.

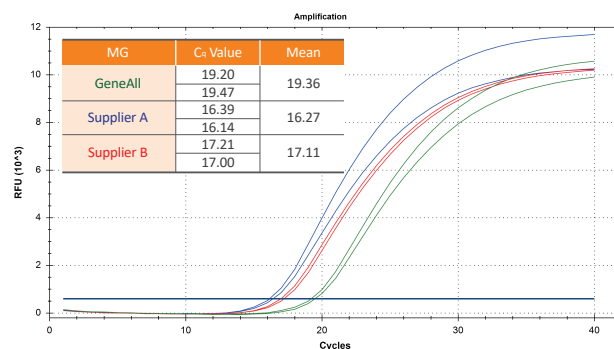
7. Repeat step 6 with the remainder of the sample.
8. Add 600  $\mu$ l of Buffer RBW to the mini column, centrifuge at  $10,000 \times g$  above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.
9. Add 600  $\mu$ l of Buffer RNW to the mini column, centrifuge at  $10,000 \times g$  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  $\mu$ l 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)