Performance evaluation of Ribospin™ Pathogen/TNA from 4 types of pathogen

Experimental Conditions

Materials Required

- Ribospin™ Pathogen/TNA (341-150)
- 1.5 ml microcentrifuge tube
- 1X PBS (Phosphate-buffered saline), pH 7.4 (SM-P04-100)
- 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)	Rabies Virus (RV)	Japanese Encephalitis Virus (JEV)
Target	Pathogen DNA/RNA			
Sample	K562 cells infected with pathogen (1 x 10^6 cells)			
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

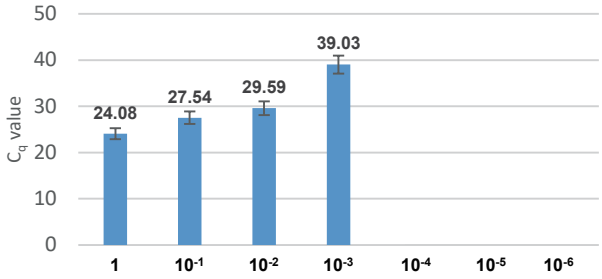
- **Proteinase K solution**
  Before start experiment, Proteinase K (24 mg) mix to 1.2 ml of PK Storage Buffer carefully to avoid foaming.

Protocol for K562 cells infected with pathogen

1. Transfer 200 µl of sample (1 x 10^6 cells in 200 µl of 1X PBS) to the 1.5 ml microcentrifuge tube. If the sample volume is less than 200 µl, adjust the volume to 200 µl with 1X PBS.
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 KL 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. 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.
8. 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.
9. Centrifuge at full speed for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.
10. Add 50 µl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.
11. Centrifuge at full speed for 1 min.

Result

Mycoplasma Gallisepticum (MG)



Infectious Bronchitis Virus (IBV)

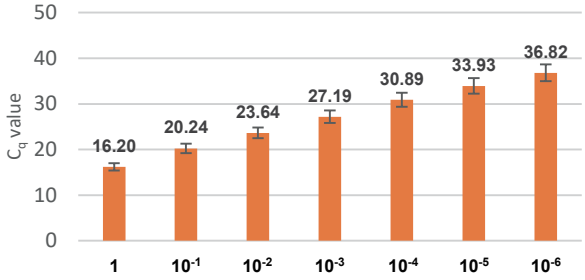


Figure 1. qPCR for the sensitivity evaluation
The samples infected with pathogens (MG, IBV) were serially diluted from 10^{-1} to 10^{-6} respectively, and TNA was extracted using Ribospin™ Pathogen/TNA. qPCR was performed to measure the sensitivity of the extraction.
• Real-time PCR system: CFX96™ System (1855201, supplier B)
• qPCR kit: RealAmp™ 2X qPCR Master Mix (801-020)
• RT-qPCR kit: HyperScript™ One-step RT-PCR Master Mix (602-110)

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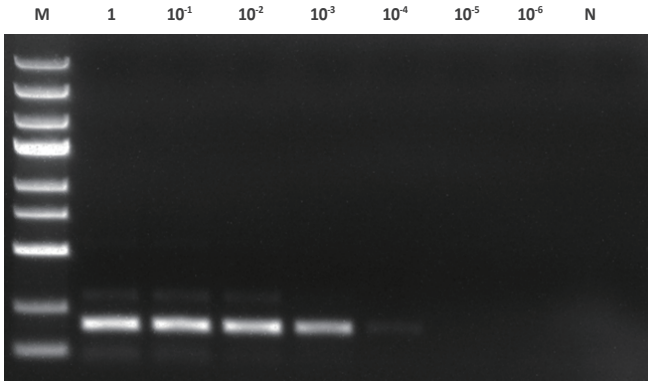
Rabies virus (RV)



Japanese encephalitis virus (JEV)

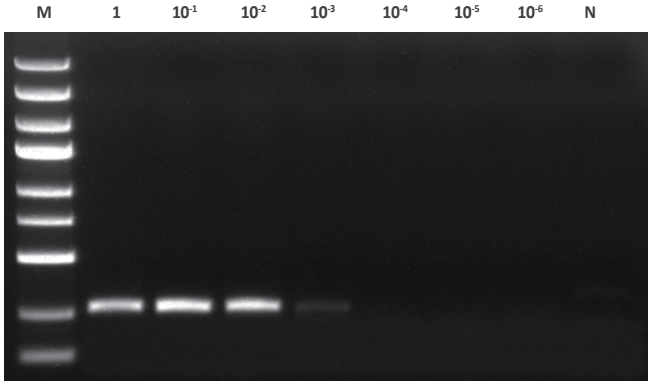


Figure 2. RT-PCR and electrophoresis for the sensitivity evaluation
The samples infected with pathogens (RV, JEV) were serially diluted from 10^{-1} to 10^{-6} respectively, and TNA was extracted using Ribospin™ Pathogen/TNA. RT-PCR and electrophoresis were performed to measure the sensitivity of the extraction.
- RT-PCR kit: HyperScript™ One-step RT-PCR Master Mix (602-110)
- PCR system: MultiGene™ Optimax Thermal Cycler (TC9610, supplier L)
- M: GENESTA™ 250 bp DNA Ladder (GA-025)
- N: negative control
- Gel electrophoresis condition: 1.2% agarose gel