A. phagocytophilum Detection Kit

For *Anaplasma phagocytophilum* Detection

User Manual

For Research Use Only

Manufacturer:

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Content

INTENDED USE

POCKIT™ *A. phagocytophilum* Detection Kit uses insulated isothermal polymerase chain reaction (iiPCR) technology to detect the DNA of *Anaplasma phagocytophilum* (Chang et al., 2012; Tsai et al., 2012). This detection kit is specially designed to be used on an iiPCR-compatible instrument, **POCKIT™** Nucleic Acid Analyzer. The intended users of the kit are veterinarians or technicians who have basic laboratory skills.

This kit is intended for research purpose and *in vitro* use only.

SUMMARY AND EXPLANATION

Anaplasma phagocytophilum (formerly *Ehrlichia phagocytophilum*) is an obligate intracellular bacterium transmitted by ticks in the family *Ixodidae*. This bacterium can infect the neutrophil granulocytes of mammals, and elicit febrile diseases in animals and human beings. *A. phagocytophilum* is also the etiologic agent of equine granulocytic anaplasmosis (EGA). Infected horses may show symptoms including fever, lethargy (tiredness), depression, anorexia, anemia, leukopenia, limb oedema and ataxia, leading to significant economic losses on the equine industry (Kroeze & Van der Kolk, 2013).

PRINCIPLES OF THE PROCEDURE

The assay is based on iiPCR for qualitative detection of *A. phagocytophilum*. Fluorogenic probe hydrolysis chemistry is used to generate fluorescent signal when a specific DNA sequence of *A. phagocytophilum* is amplified. The primers and probe only target specific sequences of *A. phagocytophilum*, and do not react with host genomic DNA and nucleic acids of other pathogens.

PRODUCT DESCRIPTION

A. Materials Provided

1) **POCKIT™** *A. phagocytophilum* Detection Kit, 48 tests/kit

Component	Contents or Purpose	Amount
Premix Pack	■ *A. phagocytophilum* Premix (lyophilized pellet) containing dNTPs, primers, probe, and enzyme for amplification. ■ Desiccating agent pack.	6 bags (8 Premix vials and 1 desiccating agent/bag)
Premix Buffer B	■ Reaction buffer to re-dissolve the lyophilized pellet.	2 vials (1.3 ml/vial)
P(+) Control	■ Dried plasmid containing *A. phagocytophilum* partial sequence as positive control.	1 vial
P(+) Control Buffer	■ Reaction buffer to re-dissolve P(+) Control.	1 vial (110 µl/vial)
User Manual		1 copy

2) **R-tube™** , 48 tubes/pack

B. Materials and Equipment Required, but Not Provided

1) **PetNAD™** Nucleic Acid Co-prep Kit or **taco™ mini** Automatic Nucleic Acid Extraction System (optional)

2) **POCKIT™** Nucleic Acid Analyzer: the iiPCR-compatible instrument.

3) **cubee™** Mini-Centrifuge

4) Micropipette and tips

C. Storage and Stability

1) The kit should be stored at 4℃ and is stable until the expiration date stated on the label.

2) Store Premix vials in sealed Premix Pack to avoid hydration of lyophilized components.

3) Reconstituted P(+) Control is stable for 6 months at 4℃. Aliquot reconstituted P(+) Control to avoid degradation of nucleic acid.

PRECAUTIONS

A. Do not open R-tube(s) after reaction to prevent any carryover contamination.

B. Perform extraction and amplification in two independent spaces to minimize contamination.

C. Do not reuse R-tube and Premix.

D. Include the P(+) Control to:

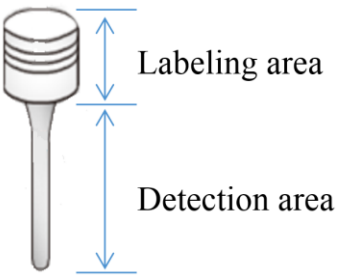
   1) Ensure **POCKIT™** Nucleic Acid Analyzer is working.

   2) Ensure detection kit performance after storage.

E. To get optimal fluorescence detection.

   1) Wear powder-free gloves to handle R-tubes.

   2) Do not label in the detection area of R-tube.



LIMITATIONS

A. The test should only be used for testing nucleic acid extracts. Do not add specimens directly into the Premix.

B. **PetNAD™** Nucleic Acid Co-prep Kit or **taco™ mini** Automatic Nucleic Acid Extraction System is recommended for nucleic acid extraction.

C. Any deviation from the recommended procedures may lead to sub-optimal results. Performance of the modified protocol should be validated by the users.

D. It is strongly recommended to use freshly prepared nucleic acids (within 1 hour after extraction) to achieve optimal results with **POCKIT™** *A. phagocytophilum* Detection Kit.

SAMPLE PREPARATION

A. Sample Type

This detection kit is intended for analyzing nucleic acids extracted from whole blood samples.

B. Using PetNAD™ Nucleic Acid Co-prep Kit

- **Note: Make sure to add 95% (or higher) ethanol to "PB2", "PB3" and "PB4" before first use.**

1) Add 200 µl whole blood samples into a clean 1.5 ml microcentrifuge tube with 600 µl PB1.
2) Mix thoroughly for 1 minute.
3) Add 600 µl PB2 (with ethanol) into the tube.
4) Mix thoroughly for 10 seconds.
5) Place a spin column onto a collection tube.
6) Transfer 600 µl of the mixture to the spin column & collection tube set.
7) Spin for 1 minute and discard the flow-through from collection tube. Reassemble the collection tube to the spin column.
8) Add 600 µl PB3 (with ethanol) into the spin column & collection tube set.
9) Spin for 1 minute and discard the flow-through.

10) Add 600 µl PB4 (with ethanol) into the spin column & collection tube set.

11) Spin for 1 minute and discard the flow-through.

12) Spin for another 3 minutes to remove residual ethanol.

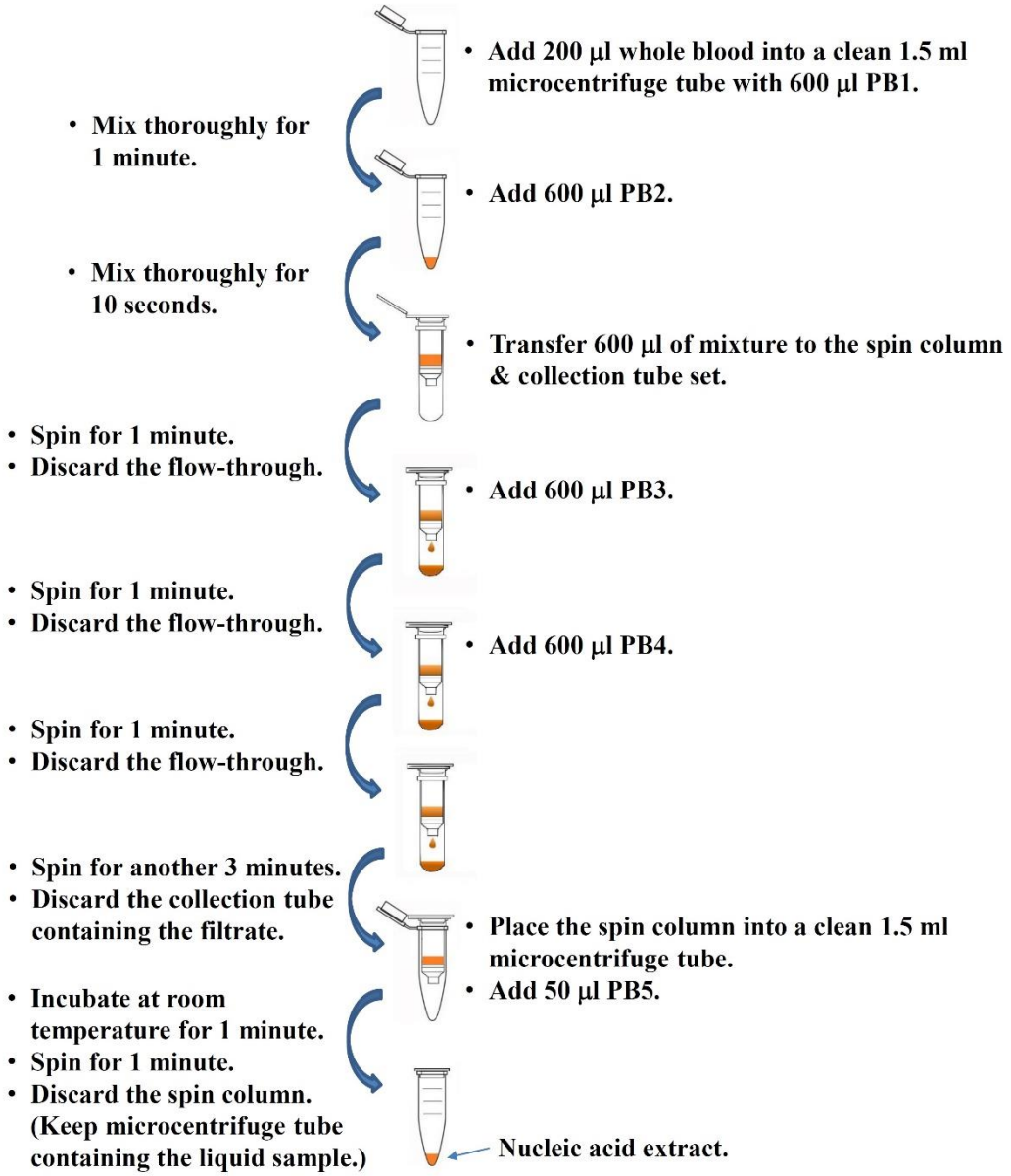
13) Transfer the spin column to a clean 1.5 ml microcentrifuge tube.

14) Add 50 µl PB5 into the spin column. Incubate at room temperature for 1 minute.

15) Spin for 1 minute to elute the nucleic acids into the 1.5 ml microcentrifuge tube.

16) Discard the spin column and proceed to iiPCR analysis of the nucleic acid extract as soon as possible.

C. Quick Guide



OPERATION PROCEDURE

A. Using POCKIT™ *A. phagocytophilum* Detection Kit

- **Note: Before preparing the reactions for iiPCR testing, turn on POCKIT™ Nucleic Acid Analyzer to initiate the calibration for the instrument. The device will complete self-test within 5 minutes. Please refer to the user manual of POCKIT™ Nucleic Acid Analyzer for further details.**

- **Note: Before using for the first time, add 100 µl P(+) Control Buffer to P(+) Control. Store reconstituted P(+) Control at 4°C.**

1) Label R-tube(s) in the labeling area.

2) Prepare one Premix for each sample. (Premix tubes are in Premix Pack. Each Premix Pack contains eight Premix tubes.)

   - **Note: When the pellet is not found at the bottom of the tube, spin tube briefly to bring it down.**

3) Add 50 µl Premix Buffer B to each Premix tube.

4) Add 5 µl nucleic acid extract or dissolved P(+) Control to each Premix tube. Spin Premix tubes briefly in a mini centrifuge (such as **cubee™** Mini-Centrifuge).

5) Transfer 50 µl Premix/sample mixture into R-tube.

6) Seal top of each R-tube with a cap. Make sure R-tube is capped tightly.

7) Place R-tube into the holder of **POCKIT™** Nucleic Acid

Analyzer.

8) Spin tube/holder set briefly in **cubee™** Mini-Centrifuge to make sure all solution is collected at the bottom of R-tube.

   - **Note: Make sure there are no bubbles in the solution.**
   - **Note: Start reaction within 1 hour to prevent nucleic acid degradation (to prevent nucleic acid degradation and non-specific reaction).**

9) **POCKIT™** Nucleic Acid Analyzer reaction:

   a) Select "520 nm".

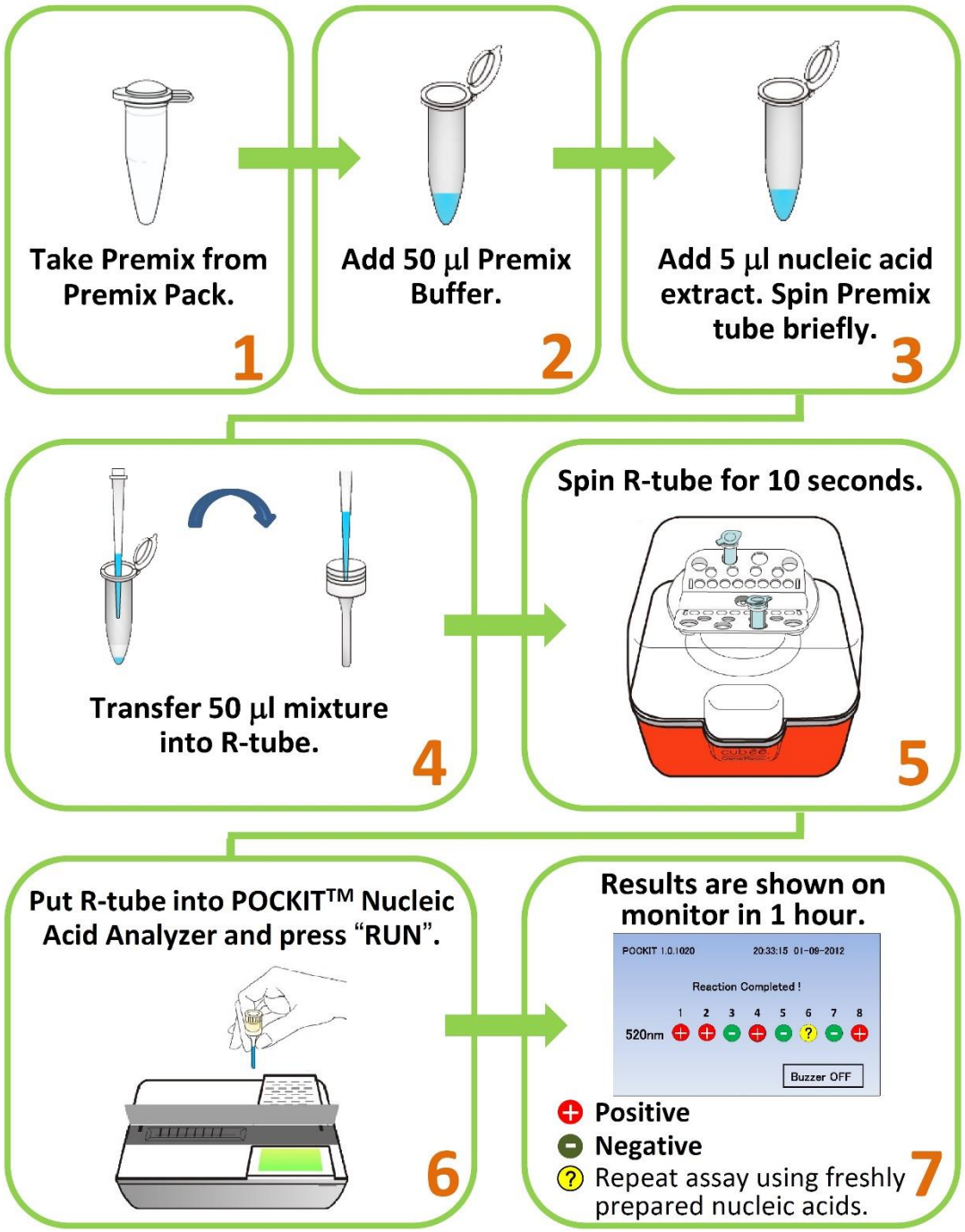
   b) When "System READY" is displayed, place the holder with R-tube(s) into the reaction chamber.

   c) Tap cap of each R-tube to make sure the tube is positioned properly.

10) Close lid and press "Run" to start reaction program.

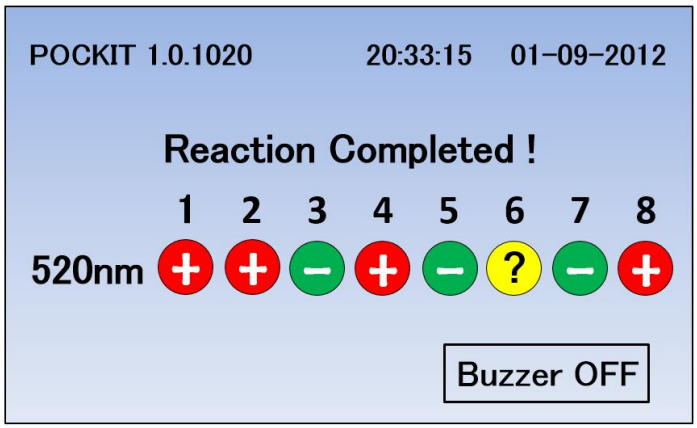
11) Test results are shown on the monitor after the reaction is complete.

B. Quick Guide



DATA INTERPRETATION

*One example of results shown on the monitor.



520 nm	Interpretation
+	*A. phagocytophilum* Positive
−	*A. phagocytophilum* Negative
?	Repeat reaction with freshly prepared nucleic acid.

ANALYTICAL SENSITIVITY

The detection limit of **POCKIT™** *A. phagocytophilum* Detection Kit is about 10 copies/ reaction.

TROUBLESHOOTING

Problems	Possible causes	Solutions
False Positive	**1)** Reuse of micro-centrifuge tubes, tips, R-tubes and Premix.	■ Micro-centrifuge tubes, tips, R-tubes and Premix are for single-use only. Reusing these accessories would cause cross-contamination. ■ Used micro-centrifuge tubes, tips, R-tubes and Premix should be collected and discarded according to local regulation. Do not place the waste close to the working area to prevent cross-contamination.
	2) Contaminated micropipette.	■ Use aerosol-free tips.
	3) Contaminated reagent.	■ Consult with a **GeneReach** technical support representative or local distributor.
	4) Leakage or spill of reaction from R-tube into reaction chamber of POCKIT™ Nucleic Acid Analyzer.	■ Consult with a **GeneReach** technical support representative or local distributor.
	5) Contaminated working area.	■ Consult with a **GeneReach** technical support representative on how to clean up working area.

Problems	**Possible causes**	**Solutions**
False Negative	**1)** Nucleic acid extraction failed.	■ Consult manual of nucleic acid extraction kit.
	2) Poor nucleic acid quality.	■ Check sample storage condition. ■ Please refer to Troubleshooting section of PetNAD™ Nucleic Acid Co-prep Kit.
	3) PCR inhibition.	■ Do not overload nucleic acid. ■ Spike 5 μl nucleic acid sample into a positive control reaction for a parallel PCR reaction. Negative results indicate the presence of inhibitors in the nucleic acid. In that case, prepare another nucleic acid extract.

REFERENCE

1. Chang, H. G., Tsai, Y., Tsai, C., Lin, C., Lee, P., Teng, P., et al. (2012). A thermally baffled device for highly stabilized convective PCR. *Biotechnology Journal*, *7*(5), 662-666. doi: 10.1002/ biot.201100453

2. Kroeze, EJB. Veldhuis., & Van der Kolk, JH (2013). *Infectious Diseases of the Horse: Diagnosis, pathology, management, and public health*. Boca Raton: CRC Press.

3. Tsai, Y., Wang, H. T., Chang, H. G., Tsai, C., Lin, C., Teng, P., et al. (2012). Development of TaqMan Probe-Based Insulated Isothermal PCR (iiPCR) for Sensitive and Specific On-Site Pathogen Detection. *PLoS ONE*, *7*(9), e45278. doi:10.1371/journal.pone.0045278