PetNAD™

Canine Tick-Borne Diseases Panel

For Canine Babesiosis,
Babesia gibsoni,
Ehrlichia canis,
and *Anaplasma platys*

User Manual

For Research Use Only

Manufacturer:

GeneReach Biotechnology Corporation

TEL: 886-4-24639869 FAX: 886-4-24638255

No. 19, Keyuan 2nd Rd., Central Taiwan Science Park, Taichung City, Taiwan 407

Web Site: www.petnad.com

2013/07

Content

INTENDED USE

PetNAD™ Canine Tick-Borne Disease Panel is intended for *in vitro* detection of Canine Babesiosis, *Babesia gibsoni*, *Ehrlichia canis* and *Anaplasma platys* based on insulated isothermal polymerase chain reaction (iiPCR) technology. This panel is designed specially to be used with an iiPCR-compatible instrument, **POCKIT™** Nucleic Acid Analyzer. The assay is intended for use by people with basic laboratory skills.

This kit is intended for research use only.

SUMMARY AND EXPLANATION

Tick-borne diseases caused by *Babesia canis*, *Babesia gibsoni Ehrlichia canis and Anaplasma platys*(formerly *Ehrlichia platys*), often occur in dog whose main vector is the brown-dog tick, Rhipicephalus sanguineus. Clinical abnormalities associated with tick-borne diseases often include lethargy, anorexia, pale mucosa membranes, haemolytic anaemia, haemoglobinuria and thrombocytopenia (Lobetti, 1998; Bourdoiseau, 2006).

PCR is one of the most commonly accepted methods that provide high sensitivity and specificity for canine tick-borne disease detection. However, conventional PCR assays take three to four hours, and require sophisticated thermocyclers and well-trained technicians to perform. GeneReach has developed **PetNAD™** Canine Tick-Borne Disease Panel based on iiPCR technology, which significantly reduces reaction time and offers sensitivity and specificity comparables to those of conventional nested PCR (Tsai, 2012; Chang, 2012). Furthermore, this simple and easy assay could be completed rapidly in a portable **POCKIT™** Nucleic Acid Analyzer.

PRINCIPLES OF THE PROCEDURE

In iiPCR, hydrolysis probe-based chemistry is used to generate fluorescent signal during amplification of target DNA. The primers and probe target specific genes and do not cross-react with nucleic acid from host and other tick-borne pathogens.

PRODUCT DESCRIPTION

A. Materials Provided (4 combo tests for 8 dogs)

Component	Contents or Purpose	Amount
Premix Pack	■ Canine Babesia Premix, *Babesia gibsoni* Premix, *Ehrlichia canis* Premix and *Anaplasma platys* Premix (lyophilized pellet) containing dNTPs, primers, probe, and enzyme for amplification. ■ Panel P(+) Standard Premix ■ Desiccating agent pack.	5 bags (8 tubes and 1 desiccating/bag)
Premix Buffer B	■ Reaction buffer to re-dissolve the lyophilized pellet.	2 vials (1.3 ml/vial)
P(+) Standard	■ Dried P(+) control template.	1 vial
Standard Buffer	■ Reaction buffer to re-dissolve P(+) Standard.	1 vial (110 µl/vial)
User Manual		1 copy

B. Materials and Equipments Required, but Not Provided

1) **PetNAD™** Nucleic Acid Co-prep Kit
2) **POCKIT™** Nucleic Acid Analyzer: **PetNAD™**-compatible instrument.
3) **cubee™** Mini-Centrifuge (cubee)
4) Micropipette and tips

C. Storage and Stability

1) The kit should be stored at 4℃ and is stable until the expiration date which is stated on the label.
2) Store Premix vials in sealed Premix Pack to avoid hydration of lyophilized components.
3) Reconstituted P (+) Standard is stable for 6 months at 4℃. Aliquot reconstituted P (+) Standard to avoid degradation and contamination of nucleic acid.

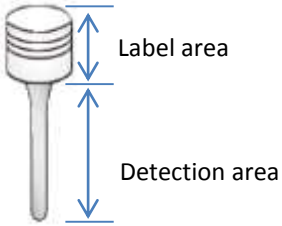
D. Sample Type

Nucleic acid extracted from whole blood.

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(+) Standard to:
    1) Ensure **POCKIT™** Nucleic Acid Analyzer is working normally.
    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.

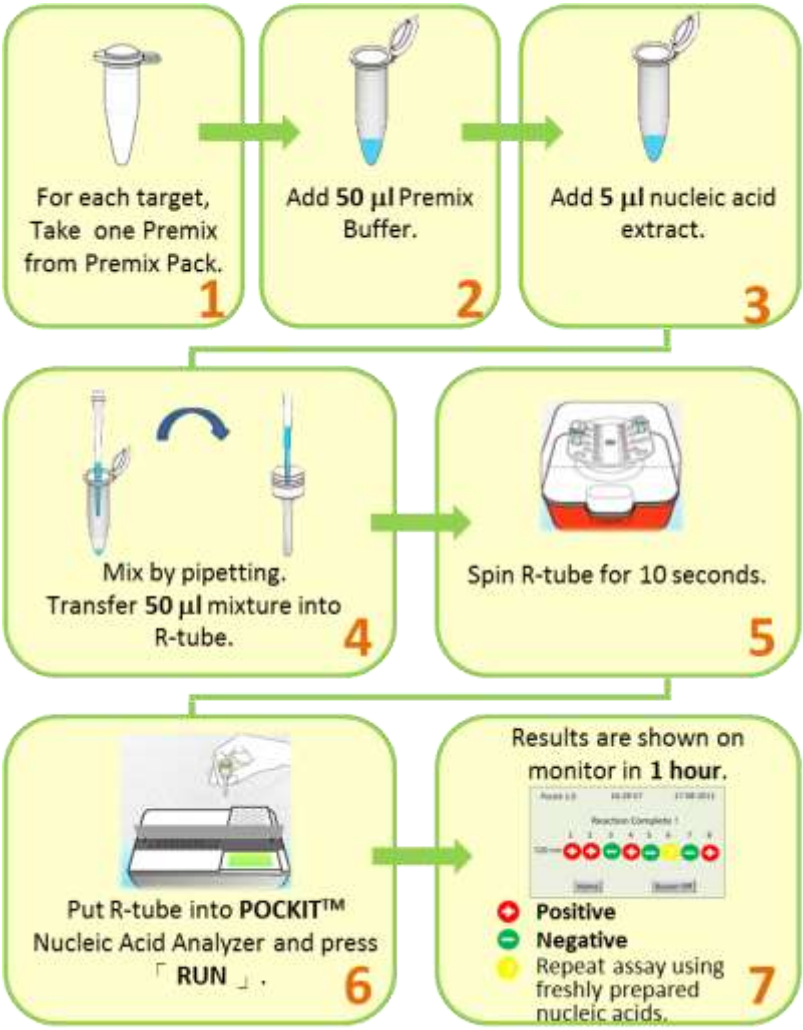


LIMITATIONS

A. The test should be used only for testing nucleic acid extracted from animal specimen. Do not add specimen (i.e. whole blood) directly into Premix.
B. **PetNAD™** Nucleic Acid Co-prep Kit is recommended for nucleic acid extraction.
C. Any deviation from recommended procedure may not achieve the optimal results and should be validated by the users.
D. It is strongly recommended to use freshly prepared nucleic acid (within 1 hour after extraction) to achieve optimal results with **PetNAD™** Canine Tick-Borne Disease Panel.

PROCEDURE

A. PetNAD™ Canine Tick-Borne Disease Panel Quick Guide



B. P(+) Standard Preparation

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

1) Label R-tube(s) in the label area.
2) Prepare one P(+) Standard Premix for each run. (Premix tube is in the Panel P(+) Standard Premix Pack, containing eight Premix tubes.)

Note: If 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 the Premix tube.
4) Add 5 µl P(+) Standard to the Premix tube. Mix by pipetting up and down.
5) Follow **Procedure C, Step 5** to proceed P(+) Standard preparation.

C. Procedure

1) Label R-tube(s) in the label area.
2) For each target, prepare **one** Premix tube. (Premix tube is in the Premix Pack. Each Premix Pack contains eight Premix tubes.)

Note: If 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 to each Premix tube. Mix by pipetting up and down.
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™**.
8) Spin tube briefly in **cubee™** to make sure all solution is collected at the bottom of R-tube.

Note: Start reaction within 1 hour to prevent nucleic acid degradation.

Note: Make sure there are no bubbles in the tube.

9) **POCKIT™** reaction:

Note: Please see the user manual of POCKIT™ for details.

   a) Turn on **POCKIT™**, which should complete self-testing within 5 minutes.
   b) Select ″520 nm″.
   c) When ″System READY″ is displayed, place the holder with R-tube(s) into the reaction chamber.
   d) 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 reaction is completed.

DATA INTERPRETATION

* One example of results shown on the monitor.

520nm	Interpretation
⊕	Positive infection
⊖	Negative infection
?	Repeat reaction with freshly prepared nucleic acid

ANYLYTICAL SENSITIVITY

The detection limit of **PetNAD™** Canine Tick-Borne Disease Panel 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	■ Disassemble and clean up micropipette. ■ Use aerosol-free tips.
	3) Contaminated reagent	■ Consult with a GeneReach technical support representative or local distributor.
	4) Contaminated working area	■ Consult with a GeneReach technical support representative on how to clean up working area.

Problems	**Possible causes**	**Solutions**
False	1) Nucleic acid	■ Consult manual of nucleic acid

Negative	extraction failed.	extraction kit.
	2) Bad nucleic acid quality or nucleic acid concentration too high	■ Check sample storage condition. ■ Please refer to Troubleshooting section of PetNAD™ Nucleic Acid Co-prep Kit. ■ If a spectrophotometer is available, check OD 260/280 ratio. This ratio should be between 1.4 and 2.0.
	3) PCR inhibition	■ Do not overload nucleic acid. ■ Spike nucleic acid sample into P(+) Standard 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.
Heavy contamination of amplicons in reaction chamber of **POCKIT™**.	1) Leakage or spill of reaction from R-tube into reaction chamber of **POCKIT™**.	■ Consult with a GeneReach technical support representative or local distributor.

REFERENCE

1. Bourdoiseau, G (2006). Canine babesiosis in France. *Vet*

Parasitol, **138:**118-125.

2. Chang, H.F. G., Tsai, Y.L., Tsai, C.F., Lin, C.K., Lee, P.Y., Teng, P.H., Su, C. and Jeng, C.C., (2012). A thermally baffled device for highly stabilized convective PCR. *Biotechnology Journal* 7(5): 662-666, doi: 10.1002/ biot.201100453
3. Harrus, S., Bark, H. and Waner, T., (1997). Canine monocytic ehrlichiosis: An update. Compendium of Continuing Education for the Veterinary Practitioner, 19 (4): 431-444.
4. Lobetti, R.G., (1998). Canine babesiosis. *Compend Cont Educ Pract Vet* 20:418-431
5. Murphy, G.L., Ewing, S.A., Whitworth L.C., Fox J.C. and Kocan, A.A. (1998). A molecular and serologic survey of *Ehrlichia canis*, *E. chaffeensis*, and *E. ewingii* in dogs and ticks from Oklahoma. Vet Parasitol 79: 325-339.
6. Tsai Y.L., Wang H.T.T., Chang H.F.G., Tsai C.F., Lin C.K., Teng P.H., Su C. and Jeng C.C., (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