PetNAD™

CDV Detection Kit

For Canine Distemper Virus

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

2013/03

Content

INTENDED USE

PetNAD™ CDV Detection Kit is intended for *in vitro* detection of CDV RNA based on insulated isothermal polymerase chain reaction (iiPCR) technology. This kit is designed specially to be used with an iiPCR-compatible instrument, **POCKIT™** Nucleic Acid Analyzer. The assay is intended for use by veterinarians or technicians with basic laboratory skills.

This kit is intended for research use only.

SUMMARY AND EXPLANATION

CDV is a single-stranded RNA virus of the paramyxovirus family. It is commonly seen in puppies of 3 to 6 months old and in young unvaccinated dogs. Infection in dogs can result in subclinical infection, gastrointestinal signs, and/or respiratory signs, frequently with central nervous system (CNS) involvement, high morbidity and mortality. Early clinical diagnosis is difficult since the initial symptoms are indistinguishable from those of the kennel cough. Serologic detection may be useful, but poses a problem in young puppies due to uncertainty caused by maternal antibody interference.

PCR is one of the most commonly accepted methods that provide high sensitivity and specificity for CDV detection. However, conventional PCR assays take three to four hours, and require sophisticated thermocyclers and well-trained technicians to perform. GeneReach has developed **PetNAD™** CDV Detection Kit 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 RNA. The primers and probe target nucleocapsid protein gene (N gene) and do not cross-react with nucleic acid from host and other canine pathogens.

PRODUCT DESCRIPTION

A. Materials Provided (24 tests/kit)

Component	Contents or Purpose	Amount
Premix Pack	■ CDV Premix (lyophilized pellet) containing dNTPs, primers, probe, and enzyme for amplification. ■ Desiccating agent pack.	24 bags (1 CDV Premix vial and desiccating agent/bag)
Premix Buffer B	■ Reaction buffer to re-dissolve the lyophilized pellet.	2 vials (1.3 ml/vial)
P(+) Standard	■ Dried plasmid containing CDV partial sequence.	1 vial
Standard Buffer	■ Reaction buffer to re-dissolve P(+) Standard.	1 vial (110 µl/vial)
R-tube		1 bag (24 pieces/bag)
Cap		1 bag (24 pieces/bag)
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 of nucleic acid.

D. Sample Type

Nucleic acid extracted from whole blood, urine and swab sample.

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:

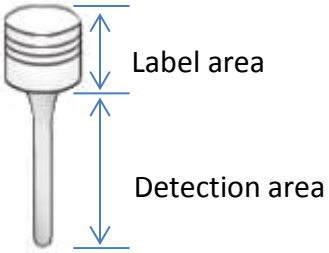
   1) Ensure **POCKIT™** Nucleic Acid Analyzer is working normally.

   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.

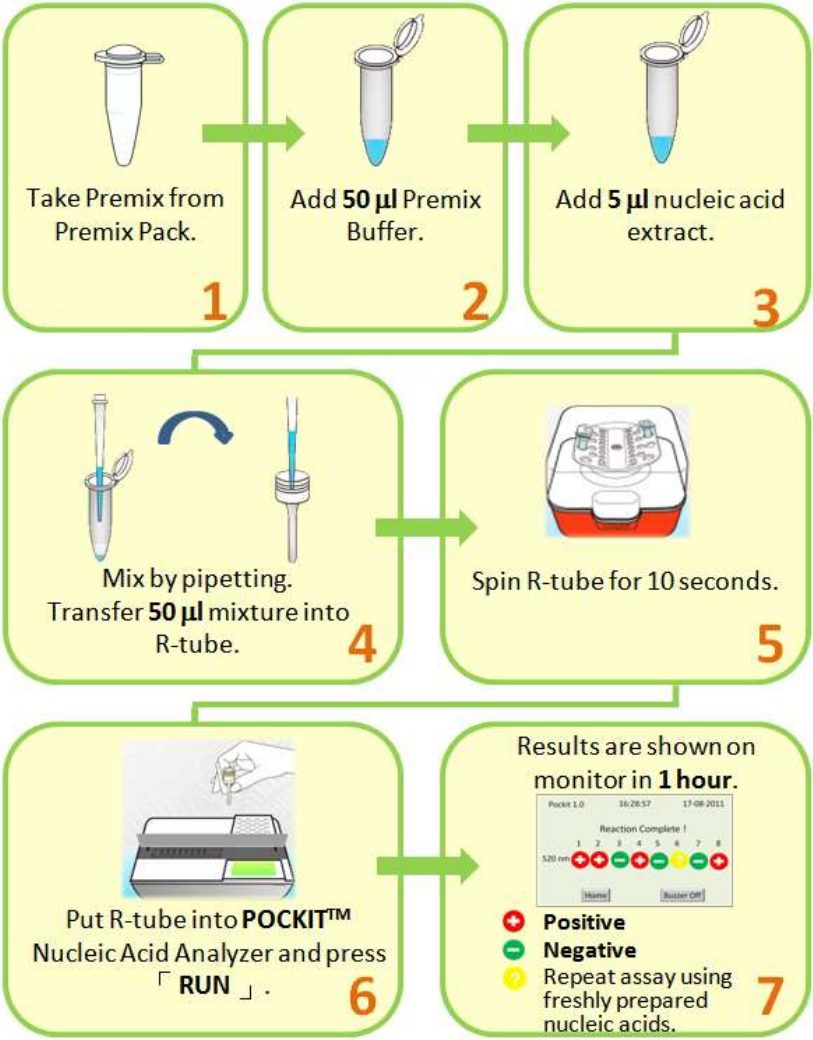
C. Any deviation from recommended procedure may not achieve the optimal results and should be validated by the users.

D. It is strongly recommended to use freshly prepared nucleic acid (within 1 hour after extraction) to achieve optimal results with **PetNAD™** CDV Detection Kit.

E. Vaccination with a modified-live CDV vaccine may result in positive PCR results for a few weeks after vaccination. Killed or vectored-recombinant vaccines will not interfere with PCR testing. PetNAD™ is recommended in sick animals with clinical signs and/or laboratory abnormalities consistent with infection or in an animal with a suspected subclinical infection as based upon history, physical examination and clinical laboratory findings.

PROCEDURE

A. PetNAD™ CDV Detection Kit Quick Guide



B. Procedure

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

1) Label R-tube(s) in the label area.
2) Prepare one Premix for each sample. (Premix tube is in Premix Pack. Each Premix Pack contains one Premix.)

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 or P(+) Standard 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
	CDV Positive
	CDV Negative
	Repeat reaction with freshly prepared nucleic acid.

ANYLYTICAL SENSITIVITY

The detection limit of **PetNAD™** CDV 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	■ 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.

Problems	**Possible causes**	**Solutions**
False Negative	1) Nucleic acid extraction failed.	■ Consult manual of nucleic acid 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. Appel, M.J.G., (1970). Distemper pathogenesis in dogs. *J Am Vet Med Assoc* 156, 1681-1684
2. Bell, S.C., Carter, S.D. and Bennett, D., (1991). Canine distemper viral antigens and antibodies in dogs with rheumatoid arthritis. *Res Vet Sci* 50, 64-68.
3. 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
4. Iwatsuki K., Miyashita, N., Yoshida, E., Gemma, T., Shin, Y.S., Mori, T., Hirayama, N., Kai, C. and Mikami, T., (1997). Molecular and phylogenetic analyses of the haemagglutinin proteins of field isolates of canine distemper virus from naturally infected dogs. *Journal of General Virology* (78): 373-380.
5. Saito, T.B., Alfieri, A.A., Wosiacki, S.R., Negrao, F.J., Morais, H.S. and Alfieri, A.F., (2006). Detection of canine distemper virus by reverse transcriptase-polymerase chain reaction in the urine of dogs with clinical signs of distemper encephalitis. *Res Vet Sci* 80, 116-119.
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
7. Vandevelde, M. and Zurbriggen, A., (1995). The neuro-biology of canine distemper virus infection. *Vet Microbiol* 44: 271-280.