PetNAD™

CDV Detection Kit

for Canine Distemper Virus

User Manual

For Research Use Only

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Content

INTENDED USE

PetNAD™ Canine Distemper Virus (CDV) Detection Kit is intended for *in vitro* detection of CDV RNA based on the insulated isothermal polymerase chain reaction (iiPCR) technology. This kit is specially designed to be used with a compatible iiPCR instrument, **POCKIT™** Nucleic Acid Analyzer.

The intended user of this kit is veterinarians or lab technicians who have basic laboratory skills.

This kit is intended for research use.

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.

CDV detection by PCR is the most sensitive and specific method. However, conventional PCR takes three to four hours and requires delicate machines as well as well-trained technicians to perform the test. GeneReach has developed **PetNAD™** CDV Detection Kit based on iiPCR technology, which significantly reduces the reaction time, and is as sensitive and specific as the conventional PCR for CDV detection. The assay has been simplified for easier and faster operation using compact equipments for CDV detection in the clinic.

PRINCIPLES OF THE PROCEDURE

The assay is based on iiPCR. In addition to specific primers, fluorogenic probe hydrolysis chemistry is used to generate a fluorescent signal when specific CDV RNA is presented in samples. The primers and probe target the nucleocapsid protein gene (N gene) specific to CDV and will not react with canine genomic DNA and nucleic acid of other pathogens.

PRODUCT DESCRIPTION

A. Materials Provided (24 tests/kit)

Component	Contents or Purpose	Amount
Premix Pack	Each pack contains 1 pack of desiccating agent and 1 Premix vial with a lyophilized pellet containing dNTPs, CDV specific primers, fluorescent probes, and enzyme.	1 zip-lock bag containing 24 individually sealed packs
Premix Buffer B	Reaction buffer to re-dissolve the lyophilized pellet	700 µl/vial, 2 vials
P(+) Standard	Dry plasmid pellet containing CDV partial sequence	1 vial
Standard Buffer	Reaction buffer to re-dissolve the CDV P(+) Standard	110 µl/vial, 1 vial
R-tube		24 Pieces/bag, 1 bag
Cap		24 Pieces/bag, 1 bag
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B. Materials and Equipments Required, but Not Provided

1) **PetNAD™** Nucleic Acid Co-prep Kit
2) **POCKIT™**: the compatible instrument for **PetNAD™**
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) Premix vials should be kept in the sealed Premix Pack to avoid the rehydration of lyophilized pellet.
3) Dissolved P(+) Standard can be stored at 4℃ for up to 6 months. To avoid the degradation of P(+) Standard, it is recommended to aliquot the dissolved P(+) Standard into several vials.

D. Sample Type

This kit is suitable for detecting nucleic acid extracted from whole blood, urine and swab sample.

PRECAUTIONS

A. Do not open the R-tube after the amplification reaction to prevent any carryover contamination.

B. We strongly recommend that the working area for extraction procedure and amplification procedure should be separated into two independent spaces to avoid any possible contamination.

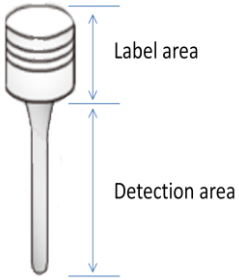
C. Do not reuse the R-tube and Premix.

D. The P(+) Standard is used to:

   1) Confirm the operation procedure after installation, or when any uncertain result has occurred;

   2) Ensure the kit performance after storage.

E. In order to get optimal fluorescence detection, please wear powder free gloves to handle the R-tube and do not mark and/or label the detection area of the R-tube. (The label area and detection area of the R-tube are indicated as shown)



LIMITATIONS

A. The test should only be used for testing nucleic acid extracted from animal specimen. Do not add specimen (i.e. whole blood) directly into the 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. Freshly prepared nucleic acid samples (within 1 hour after extraction) are strongly recommended to be used for **PetNAD™** CDV Detection Kit to achieve optimal results.

OPERATION PROCEDURE

A. PetNAD™ CDV Detection Kit Quick Guide

B. Procedure

Note: Please dissolve the P(+) Standard by 100 µl Standard Buffer at first time use. The dissolved P(+) Standard should be stored at 4℃.

1) Open the Premix Pack according to the sample number and take out the Premix.

Note: If the pellet is not at the bottom, please spin it down.

2) Open the cap, add 50 µl Premix Buffer B into each Premix tube.
3) Add 5 µl nucleic acid extract or dissolved P(+) Standard into each Premix tube. Mix by pipetting up and down.
4) Transfer 50 µl of the Premix mixture into the R-tube.
5) Cap the R-tube, put into the holder of **POCKIT™**, and use cubee to spin down the solution.

Note: Please make sure all solution has been spun down to the bottom of the R-tube. Perform the following amplification reaction within 1 hour to prevent nucleic acid degradation.

Note: Please make sure there is no bubble in the tube. Please see the user manual of POCKIT™ for details.

6) Turn on **POCKIT™**. The analyzer will complete self-testing within 5 minutes. Select 520 nm for use. “System READY” will be displayed.

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

7) Place the holder containing the R-tube(s) into the reaction chamber of **POCKIT™**, and tap the cap of each R-tube to make sure the tube is properly positioned in the reaction chamber.
8) Close the lid and press “Run” to start the reaction program.
9) The test result will be shown on the screen after the reaction.

DATA INTERPRETATION

Please check the results on the screen after the reaction.

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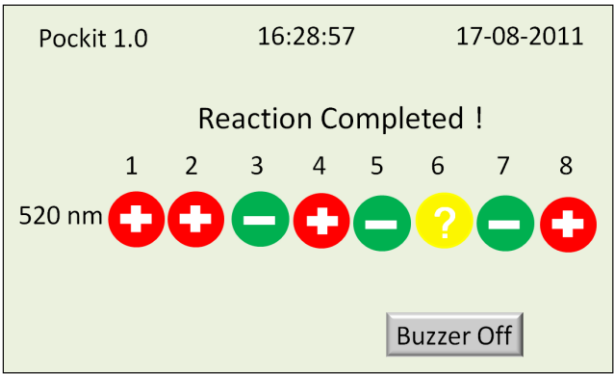
* For example, from the screen,



520nm	Interpretation
	CDV Positive
	CDV Negative
	Recheck with fresh sample.

ANYLYTICAL SENSITIVITY

The detection limit of **PetNAD™** CDV Detection Kit is up to 10 copies/ reaction.

TROUBLESHOOTING

Observation or Problems	Possible Causes	Comment and Suggestions
False Positive	**1)** The reuse of micro-centrifuge tubes, tips, R-tubes and Premix.	■ The micro-centrifuge tubes, tips, R-tubes and Premix are for one-time use only. Reuse of these accessories will cause contamination. ■ Once used, the micro-centrifuge tubes, tips, R-tubes and Premix should be collected and discarded according to the local regulation. Do not place the waste close to the working area to prevent contamination.
	2) Micropipette contaminated	■ Disassemble pipette and do clean up. We recommend using aerosol free tips.
	3) Reagent contaminated	■ Consult with GeneReach or local distributor.
	4) Working area contaminated	■ Consult with GeneReach for working area clean up.

Observation or Problems	Possible Causes	Comment and Suggestions
False Negative	**1)** Nucleic acid extraction failed.	■ Check nucleic acid extraction procedure.
	2) Bad nucleic acid quality or nucleic acid concentration too high	■ Please check the sample storage condition. ■ Please refer to the Troubleshooting section of **PetNAD™** Nucleic Acid Co-prep Kit. ■ If a spectrophotometer is available, check OD 260/280 ratio. Normally, this ratio should be 1.4 to 2.0.
	3) PCR inhibition	■ Do not add too much nucleic acid. Please follow the operation procedure. ■ Spike P(+) Standard for a parallel PCR reaction. If the one with P(+) Standard showed positive, then the inhibition was ruled out. If P(+) Standard was negative, then there was inhibition. User need to prepare another nucleic acid extraction.

Observation or Problems	**Possible Causes**	**Comment and Suggestions**
Solution or other interferences fall into the reaction chamber of POCKIT™	R-tube broken or solution spilled in the reaction chamber of **POCKIT™**	■ Consult with GeneReach or your 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., Bennett, D., 1991, Canine distemper viral antigens and antibodies in dogs with rheumatoid arthritis. Res Vet Sci 50, 64-68.
3. Iwatsuki K., Miyashita N., Yoshida E., Gemma T., Shin Y.S., Mori T., Hirayama N., Kai C.,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.
4. Saito, T.B., Alfieri, A.A., Wosiacki, S.R., Negrao, F.J., Morais, H.S., 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.
5. Vandevelde, M., Zurbriggen, A., 1995, The neuro-biology of canine distemper virus infection. Vet Microbiol 44: 271-280.