PetNAD™

Nucleic Acid Co-prep Kit

User Manual

For Research Use Only

Manufacturer:

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Content

INTENDED USE

The product is intended to generate competent DNA or RNA extract served as the template for amplification or reverse transcription respectively for **PetNAD™** detection kits. The intended users of the product shall be veterinarians (vet) or vet assistants.

The kit is for research use only.

SUMMARY AND EXPLANATION

PetNAD™ Nucleic Acid Co-prep kit is using a spin column based DNA/RNA co-extraction system. It is designed to extract DNA and RNA simultaneously in an easy and quick way from samples of canine and feline without utilizing any organic solvent.

PRODUCT DESCRIPTION

A. Materials Provided (50 tests/kit)

Item	Volume	Note: Before first use
PB1	36 ml/bottle, 1 bottle	
PB2	1 ml/bottle, 1 bottle	Add 35 ml 95% ethanol before use
PB3	20 ml/bottle, 1 bottle	Add 20 ml 95% ethanol before use
PB4	15 ml/bottle, 1 bottle	Add 25 ml 95% ethanol before use
PB5	12 ml/bottle, 1 bottle	
Spin Column & Collection Tube	50 sets/bag, 1 bag	
User Manual	1 copy	

*** Before first use, please add appropriate volume of ethanol as indicated.**

B. Materials and Equipments Required, but Not Provided

1. Micropipette and tips
2. **cubee™** Mini-centrifuge (cubee)
3. Vortex mixer
4. Micro-centrifuge tubes
5. 95% ethanol

STORAGE AND STABILITY

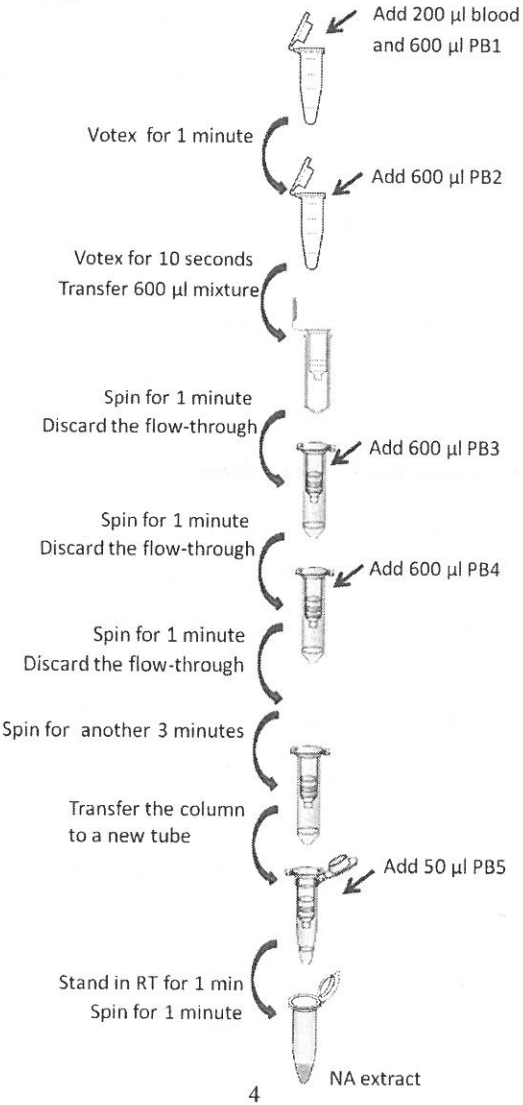
All reagents should be kept sealed tightly in cool and dry place at room temperature.

PRECAUTIONS

A. When working with chemicals, always wear suitable lab coat and disposable gloves.
B. Make sure to add adequate volume of 95% ethanol to PB2, PB3 and PB4 before first use. Mark the reagent bottle after the addition of ethanol.
C. After adding ethanol, please make sure the bottles are tightly sealed after each use.
D. The kit is used to generate DNA and RNA simultaneously from samples of canine and feline. We strongly recommend using the freshly prepared nucleic acid for downstream applications to avoid possible nucleic acid degradation.
E. Do not reuse the spin columns, collection tubes and tips.
F. The working area for extraction procedure and amplification procedure should be separated in two independent spaces to avoid any possible contamination.
G. Any deviation from the instructions may influence the kit performance and must be validated by the users.

OPERATION PROCEDURE

A. Quick Guide



B. Procedure

Note: Add adequate volume of 95% ethanol to PB2, PB3 and PB4 before first use

Note: Preserve the blood in a tube containing EDTA if it is not for immediate use.

1. Collect **fresh blood** in a tube containing EDTA, then transfer 200 µl fresh blood to a 1.5ml micro-centrifuge tube.

   Note: For other sample types please see Appendix.

2. Add 600 µl PB1 then vortex 1 minute.

   Note: For users who don't have a vortex machine, quickly shake the tube with mixture up and down to achieve the same performance.

3. Add 600 µl PB2 (with ethanol) then vortex 10 seconds.
4. Transfer 600 µl of mixture to the column.
5. Spin at full speed for 1 minute. Discard the flow-through.
6. Add 600 µl PB3 (with ethanol) into the column.
7. Spin at full speed for 1 minute. Discard the flow-through.
8. Add 600 µl PB4 (with ethanol) into the column.
9. Spin at full speed for 1 minute. Discard the flow-through.
10. Spin at full speed for another 3 minutes to remove residual ethanol.
11. Place the column in a clean 1.5 micro-centrifuge tube.
12. Add 50 µl PB5 into the column. Incubate at room temperature for 1 minute.

    Note: The volume of PB5 is subjected to changes for different sample types. Please check the user manual of specific iiPCR detection kit for instruction.

13. Spin at full speed for 1 minute to elute the nucleic acid. The extracted

nucleic acid is recommended for immediate use; otherwise it should be kept at 4˚C for 1 hour.

TROUBLESHOOTING

Observations or Problems	Possible causes	Comment and Solutions
False positive result in downstream PetNAD™ detection tests	1. The reuse of micro-centrifuge tubes, tips or spin columns.	■ The micro-centrifuge tubes, tips and spin columns are for one-time use only. Reuse of these accessories will cause contamination, and therefore false positive results. ■ Once used, the micro-centrifuge tubes, tips and spin columns 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. Reagents or working area contaminated	■ Consult with GeneReach or local distributor.

Observation or Problems	Possible Causes	Comment and Suggestions
Failure in downstream PetNAD™ detection tests	**1.** Damaged reagent bottles or spin columns	■ Check the kit components for any damage after receiving the kit. Contact GeneReach or your local distributor if the component is damaged. ■ Do not use the damaged components since their use may lead to poor kit performance.
	2. Bad sample quality	■ Please check the sample storage condition and the expiration date of the **PetNAD™** Nucleic Acid Co-prep Kit. ■ Using fresh sample for extraction is recommended. Poor sample quality may influence test result.
	3. Incorrect preparation of the reagent	■ Check to ensure the correct volume of 95% ethanol is added to PB2, PB3 and PB4 (See Material Provided).

Observation or Problems	Possible Causes	Comment and Suggestions
	4. Inappropriate operation environment	■ Operation temperature too high or low may lead to low yield of nucleic acid. We recommend users to operate **PetNAD™** Nucleic Acid Co-prep Kit under room temperature (16~30°C).
	5. Incorrect sample volume	■ Please see Operation Procedure for correct volume. ■ For sample types other than blood, please see **Appendix** for sample volume and preparation.
No flow-through	1. A clot of blood or tissue stock on the column	■ Please check the sample storage condition. ■ Repeat the extraction with new spin column and new sample.
	2. Centrifuge force is not enough	■ Please use cubee or high speed centrifuge to spin.

APPENDIX

A. Sample Type

- Blood sample: Please see the **Operation Procedure** for instructions.

- Swab sample
  i. Collect swab sample from patient.
  ii. Place the swab in a tube with 1 ml DEPC water or saline water.
  iii. Mix the swab sample and solution.
  iv. Discard the swab, and transfer 200 μl mixture for extraction (See Operation Procedure).

- Urine sample
  i. Collect urine sample from patient.
  ii. Transfer 200 μl urine to a micro-centrifuge tube for extraction (See **Operation Procedure**).

Note: We strongly recommend using fresh sample for extraction. Preserve the nucleic acid at 4°C for 1 hour after sample preparation if it is not for immediate use.