

1. DESCRIPTION

Neospora caninum is a recently discovered, apicomplexan, coccidial protozoan that causes abortion in many mammals including cattle, goats, horses and sheep. Some evidence also indicates association of this organism with neonatal neurological and neuromuscular disease in mammals such as dogs, cattle, sheep and deer.

N.caninum-induced bovine abortion has been reported in many countries including the United States, Mexico, Canada, western Europe, Central and South America, Australia and Japan. N. caninum is a major cause of bovine abortion in USA. Prospective and retrospective studies show that 20-45% of bovine abortions in drylot dairies in California were attributable to neosporosis.

In adult cattle infected with this parasite, abortion seems to be the only clinical sign. Bovine fetuses from three months to nine months of gestational age can be infected with this parasite, with most cases occurring between the fifth and seventh month of gestation. Infected calves may be born clinically normal or with neurological signs such as weakness and ataxia.

VetPCR™ NEO.CANI Detection Kit is the direct detection of *Neospora caninum* on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 3 hours for detection. Therefore, it is a very fast, accurate, reliable technique.

2. STORAGE

The components of VetPCR™ NEO.CANI Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

	Kit 48	Kit 96	
VetPCR™ NEO.CANI PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
NEO.CANI PCR Positive control	1	1	vial
NEO.CANI PCR Positive control Pre-mixture.....	4	8	tubes
Brig™ Molecular Weight marker	1	1	vial
Mineral Oil	1	1	vial
DNA purification kit (see step 6.1)	50	100	tests

4. SPECIMEN

0.5 ml whole blood in EDTA (purple top) tube, or egg (albumen), thymus or spleen.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- Pipettes
- Sterile pipette tip
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler
- Electrophoresis kit
- UV transilluminator

6. PROCEDURE

Please read through the entire procedure before starting.

6.1 DNA PREPARATION

Various manufacturers offer DNA isolation kits. Please carry out the DNA isolation according to the manufacturers instructions. The following standard DNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Genomic DNA Purification Kit (50 test)	230040(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Genomic DNA Purification Kit (100 test)	230040(100)	Bioingentech Biotechnology Inc.

6.2 AMPLIFICATION

1.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

2.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

3.- Add 2µl of template DNA into the PCR Premix tube to total volume as 13µl.

4.- Add 6µl of DNase/RNase-free water and 2µl of PCR Positive control into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11µl). This step is necessary, even when using a thermal cycler that employs a top heating method.

6.- Perform PCR reaction of samples as the below process, using a PCR thermal cycler.

PCR cycle		Temp.	Time
30 Cycles	1 Cycle	Initial Denaturation	94°C 2 min.
	30 Cycles	Denaturation	94°C 30 sec.
		Annealing	55°C 30 sec.
1 Cycle	Extension	72°C 30 sec.	
	1 Cycle	Final extension	72°C 5 min.

6.3 DETECTION OF AMPLIFIED PRODUCTS

1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).

2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

3.- Run electrophoresis by 100V (required about 30~40 minutes).

4.- Identify the result on ultra-violet (UV) transilluminator.

6.4 INTERPRETATION

- Expected PCR product size : 287 bp

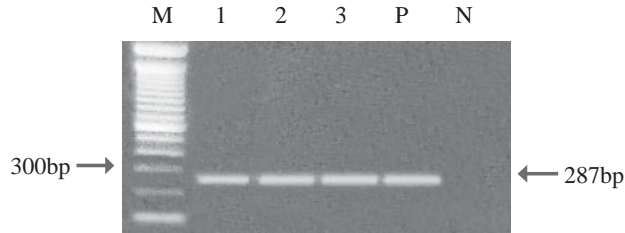


Fig1. Electrophoresis of PCR product by VetPCR™ NEO.CANI Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane 1~3 : NEO.CANI Positive sample
 Lane P : Positive control
 Lane N : Negative control

7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. Bioingentech Biotechnology Inc. does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

8. TROUBLE SHOOTING

- 1.- In the case of difficult to interpret results due to non-specific bands; reduce amount of template by 1/10 dilution, heated at 65° C for 5 min. and reacts again.
- 2.- Preparation of PCR reaction at room temperature may cause the non-specific band.
- 3.- All procedure should be carried out on ice.

9. ORDERING INFORMATION

Product	Catalog No.
VetPCR™ NEO.CANI Detection Kit 48	VET0012B(48)
VetPCR™ NEO.CANI Detection Kit 96	VET0012B(96)
Brig™ Molecular Weight Marker	24012



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