

## 1. DESCRIPTION

Canine herpesvirus (CHV) can cause fading puppy syndrome, upper respiratory tract disease (kennel cough) and abortion/stillbirths in dogs. The main route of transmission appears to be oronasal from infected puppies or from nasal or vaginal excretions of adults. The virus spreads rapidly through kennels but usually only causes disease in very young puppies. Infection of newborn puppies commonly results in death. Puppies infected with CHV at the time of birth will generally start to show clinical signs of infection at four to six days of age. Infected puppies will exhibit persistent cry, a diminished suckling response, yellow green diarrhea and abdominal pain. Fever is usually not present. Death frequently occurs within 48 hours after clinical signs are noted. One or all pups in a litter infected at birth may show signs of herpesvirus infection. Infection of adults or puppies over 3 weeks old results in replication in the respiratory tract without clinical disease. The virus can undergo latent infection and reactivation, and further shedding can be induced by immunosuppression or stress.

VetPCR™ CHV Detection Kit is the direct detection of *Canine herpesvirus* 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 and reliable technique.

## 2. STORAGE

The components of VetPCR™ CHV 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™ CHV PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
CHV PCR Positive control	1	1	vial
CHV 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

Vesicular, nasopharyngeal, conjunctival or throat swab, or 1 ml tracheal wash, or 0.5 ml whole blood in EDTA (purple top) tube, or tissue.

## 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
1 Cycle	Initial Denaturation	94°C	2 min.
30 Cycles	Denaturation	94°C	30 sec.
	Annealing	56°C	30 sec.
	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).

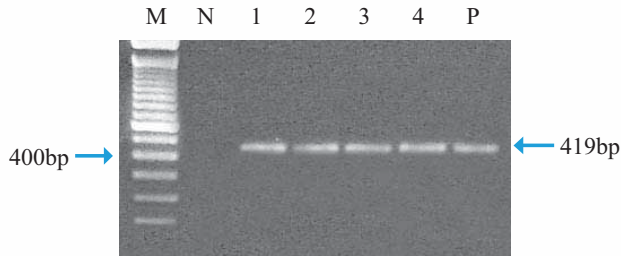
2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

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 : 419 bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ CHV Detection Kit  
Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
Lane P : Positive control  
Lane 1~4 : CHV Positive sample  
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™ CHV Detection Kit 48	VET0012C(48)
VetPCR™ CHV Detection Kit 96	VET0012C(96)
Brig™ Molecular Weight Marker	24012



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