

1. DESCRIPTION

Infectious laryngotracheitis virus (ILTV) causes a serious, worldwide-occurring respiratory disease of chickens which affects growth and egg production and may lead to death of the animals. The acute phase of infection lasts between 1 and 2 weeks and is often associated with clinical signs like gasping, coughing, expectoration of bloody mucus, and conjunctivitis. Subsequently, an asymptomatic latent infection of the central nervous system can be established. For prevention of disease chickens are immunized with attenuated live-virus vaccines that are suitable for mass application via eye drop, aerosol, or drinking water. Infectious laryngotracheitis is a contagious respiratory disease which is characterized by gasping, neck extension and conjunctivitis (inflammation of the membrane around the eye). ILTV, also designated as gallid herpes virus 1, has been classified as a member of the Alphaherpesvirinae subfamily of the Herpesviridae and represents the only species presently in the genus Infectious Laryngotracheitis-like Viruses.

VetPCR™ ILTV Detection Kit is the direct detection of Infectious Laryngotracheitis Virus 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™ ILTV 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™ ILTV PCR Pre-mixture	48	96	tubes
DNase/RNase-free water	1	1	vial
ILTV PCR Positive control	1	1	vial
ILTV 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

Tracheal swab.

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	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
	Annealing	55°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 : 666 bp

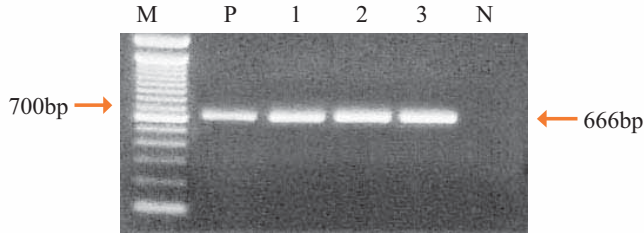


Fig1. Electrophoresis of PCR product by VetPCR™ ILTV Detection Kit
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)
 Lane P : Positive control
 Lane 1~3 : ILTV Positive sample
 Lane N : Negative control

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™ ILTV Detection Kit 48	VET0010A(48)
VetPCR™ ILTV Detection Kit 96	VET0010A(96)
Brig™ Molecular Weight Marker	24012



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