

## 1. DESCRIPTION

*Trichomonas*, a parasitic flagellated protozoan, have three to five anterior flagella, an undulating membrane, and a recurrent flagellum attached to the edge of the undulating membrane.

*Trichomonas gallinae* causes avian trichomoniasis. When present, it is usually found in the upper digestive tract of many species of doves and gallinaceous birds. Some strains may also produce liver and lung lesions. The parasite is transferred to young from the mother during feeding. Transmission between birds may also occur from contaminated feed and water. Infection by this trichomonad can be fatal. The presence of this organism in doves is a common source of infection of falcons and hawks feeding on them. In addition to showing signs of general illness, for example lethargy and fluffed-up plumage, affected birds may drool saliva, regurgitate food, have difficulty in swallowing or show laboured breathing. Finches are frequently seen to have matted wet plumage around the face and beak. In some cases, swelling of the neck may be visible from a distance. The disease may progress over several days or even weeks, consequently affected birds are often emaciated.

VetPCR™ TRICH.GAL Detection Kit is the direct detection of *Trichomonas gallinae* 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™ TRICH.GAL 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™ TRICH.GAL PCR Pre-mixture.....	48	96	tubes
DNase/RNase-free water .....	1	1	vial
TRICH.GAL PCR Positive control .....	1	1	vial
TRICH.GAL 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

Cloacal, oral or other mucous secretion swab, or 0.5 ml fecal specimen. Less preferred specimens: 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.
	Denaturation	94°C	30 sec.
	Annealing	55°C	30 sec.
30 Cycles	Extension	72°C	30 sec.
	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

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

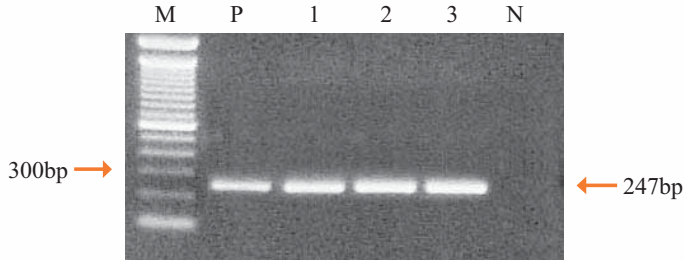
2.- Load 7µl of PCR product, 7µl of positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

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 : 247 bp



**Fig1.** Electrophoresis of PCR product by VetPCR™ TRICH.GAL Detection Kit  
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
 Lane P : Positive control  
 Lane 1~3 : TRICH.GAL 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™ TRICH.GAL Detection Kit 48	VET0018A(48)
VetPCR™ TRICH.GAL Detection Kit 96	VET0018A(96)
Brig™ Molecular Weight Marker	24012



**Bioingentech Ltd.**

Salas 350, piso 2, Concepción, Chile  
 Telephone (56)-(41)-2790435  
 Fax (56)-(41)-2790435  
 info@bioingentech.com  
 www.bioingentech.com

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