ISO 9001/14000 certified

For research use only

Cat. No. INT-IP12666 | **48 Tests**

Transmissible gastroenteritis & Porcine epidemic diarrhea virus Detection Kit

Test for the detection of Transmissible Gastroenteritis (TGE) and/or Porcine Epidemic Diarrhea (PED) by one-step RT-PCR

User Manual

REV.2.2

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Table of Contents

TGE/PED Detection Kit

■ **NOTE :**

7. NOTICE

- This product was designed to detect more than 100 copies of target gene(or gene segment). When the copy number of target present in the test reaction is less than 100, a false-negative(a negative test result when the attribute for which the subject is being ested actually exists in that subject) may occur. Use this product For Research Use Only.
- Do not use any reagent after the expiration date.
- Do not use together with reagents of other products.
- Follow the instructions.
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. iNtRON 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 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.
TGE/PED Detection Kit	IP12666
Viral Gene-spin™ Viral DNA/RNA Extraction kit	17151
SiZer™ 100 DNA Marker	24073

1. DESCRIPTION

Transmissible gastroenteritis (TGE) is a common viral disease of the small intestine that causes vomiting and profuse diarrhea in pigs of all ages. The causal TGE virus infects and destroys villous epithelial cells of the jejunum and ileum, which results in severe villous atrophy, malabsorption, osmotic diarrhea, and dehydration.

Porcine epidemic diarrhea (PED) virus diarrhea affects pigs of all ages and clinically resembles TGE in several respects. Oral infection results in viral replication in the epithelial cells of the small intestinal villi. Cells on colonic villi also become infected. No other tissue tropisms have been shown. Virus is excreted in the feces.

TGE and PED are highly contagious enteric diseases of piglets. The clinical signs of these diseases are very similar and include watery, yellowish diarrhea.

TGE/PED Detection Kit is direct detection of TGE and/or PED on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the RT-PCR (Reverse Transcription-Polymerase Chain Reaction) method, and take only 2~3 hours for detection. Therefore, it is a very fast accurate, reliable technique.

2. STORAGE

The components of ***TGE/PED Detection Kit*** should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. CONTENTS

TGE/PED RT-PCR Pre-mixture ····· 48 tubes
DNase/RNase-free water (white cap) ····· 1 vial
TGE/PED positive control (Yellow cap) ····· 1 vial

Component in 20㎕ reaction
OptiScript™ RT System
RT-PCR buffer
dNTPs
i-StarTaq™ DNA Polymerase
Chemical stabilizer
8-MOP (dissolved in DMSO)
Primers for TGE/PED

4. SPECIMEN

Performs the test with feces, intestine, lung or nasal swabs. The specimen should be stored at -20℃ prior to use.

5. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- RNA extraction kit (see 6.1 RNA preparation method)
- 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 RNA Preparation

Various manufacturers offer RNA isolation kits. Please carry out the RNA isolation according to the manufacturer's instructions. The following standard extraction kit is recommended.

Product	Catalog No.	Manufacturer
Viral gene-spin™ Viral DNA/RNA Extraction Kit	17151	iNtRON Biotechnology, Inc.

6.2 Amplification

① Prepare appropriate RT-PCR premix tubes and label. And one RT-PCR premix tube for positive control.
② Add 2㎕ of template RNA into the RT-PCR premix tube.
③ Add 18㎕ of DNase/RNase-free water into the RT-PCR premix tube to total volume as 20㎕.
④ Add 2㎕ of positive control and 18㎕ of RNase-free water into a RT-PCR premix tube for monitoring of amplification and easy interpretation.
⑤ Dissolve the blue pellet by pipetting.
Note :The pellet is easily dissolved, by letting the mixture stand at R.T. for 1-2minutes after adding water.
⑥ (*Optional)* Add mineral oil. This step is unnecessary when using a thermal cycler that employs a top heating method (general methods).
⑦ Perform PCR reaction of samples as the below process using PCR machine.

RT-PCR cycle		Temp.	Time
1 Cycle	Reverse transcription reaction	45℃	30 min.
	Inactivation of reverse transcriptase	94℃	5 min
40 Cycles	Denaturation	94℃	30 sec.
	Annealing	50℃	30 sec.
	Extension	72℃	40 sec.
1 Cycle	Final extension	72℃	5 min.

6.3 Detection of Amplified Products

① Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
② Load 7㎕ of PCR product and positive control 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 TGE PCR product size : **755 bp**
- Expected PED PCR product size : **525 bp**

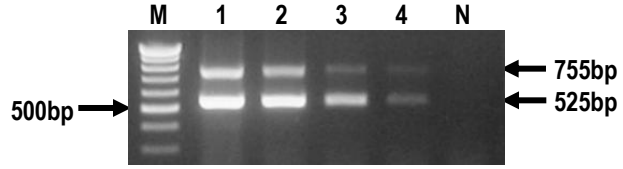

Fig 1. Electrophoresis of PCR product by **TGE/PED Detection Kit**
Lane M : 100bp molecular ladder (iNtRON Biotechnology)
Lane 1~4 : TGE/PED positive sample
Lane N : Negative control

6.5 Elimination of carry-over contamination

- Each PCR/RT-PCR Pre-mixture contains 8-methoxypsoralen (8-MOP) for preventing of carry-over contamination.
- All PCR products should be discarded after UV irradiation (10 min/365nm) for preventing from carry-over contamination.