

1. DESCRIPTION

Swine influenza is an acute, highly contagious, respiratory disease that results from infection with type A influenza virus. It is commonly known in the industry as Swine Flu, Hog Flu, and Pig Flu. The etiology of Hog Flu is a Type A Influenza virus which belongs to the *Orthomyxoviridae* family. It is an enveloped, RNA virus with a lipid envelope and a helical nucleocapsid. Field isolates of variable virulence exist, and clinical manifestation may be determined by secondary organisms. Pigs are the principal hosts of classic swine influenza virus. Influenza is transmitted primarily pig-to-pig by the nasopharyngeal route. Nasal secretions are laden with virus during the acute febrile stage. The virus is easily carried and spread by avian species, particularly waterfowl, and humans. Care should be taken to prevent spread from and between birds and humans to swine. The virus can be shed for 30 days post-inoculation and has been recovered from clinically normal animals.

VetPCR™ SIV Detection Kit is the direct detection of *Swine influenza 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™ SIV 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™ SIV RT-PCR Pre-mixture.....	48	96	tubes
VetPCR™ SIV PCR Pre-mixture	48	96	vial
BrigRT-PCR™ solution	1	1	vial
Biotech™ Transcriptase solution	1	1	vial
DNase/RNase-free water	1	1	vial
SIV RT-PCR Positive control	1	1	vial
SIV RT-PCR Positive control Pre-mixture	4	8	tubes
SIV PCR Positive control Pre-mixture	4	8	tubes
Brig™ Molecular Weight marker	1	1	vial
Mineral Oil	1	2	vial(s)
RNA extraction kit (see step 6.1)	50	100	tests

4. SPECIMEN

Deep nasal swab, trans-tracheal wash, lung or whole blood in EDTA (purple top) tube.

5. ADDITIONAL REQUIRED MATERIALS

- 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 manufacturers instructions. The following standard RNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Total RNA Purification Kit (50 test)	230041(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Total RNA Purification Kit(100 test)	230041(100)	Bioingentech Biotechnology Inc.

6.2 AMPLIFICATION

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

2.- Add 5µl of DNase/RNase-free water into the RT-PCR Premix tube to total volume as 8,5µl.

3.- Add 1,5µl of template RNA into the RT-PCR Premix tube to total volume as 10µl.

4.- Add 5µl of DNase/RNase-free water and 1,5µl of RT-PCR Positive control into a RT-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 RT-PCR reaction (RT-PCR 1) of samples as the below process using a PCR thermal cycler.

7.- Add 0,3µl of BrigRT-PCR™ solution and 0,5µl of Biotech™ Transcriptase solution.

8.- Perform RT-PCR reaction (RT-PCR 2) of samples as the below process, using a PCR thermal cycler.

		RT-PCR cycle		Temp.	Time
RT-PCR 1	1Cycle	Initial Denaturation		80°C	10 min.
	1Cycle	Stop		4°C	5 min.
Add 0,3µl of BrigRT-PCR™ and 0,5µl of Biotech™ Transcriptase					
RT-PCR 2	1Cycle	Denaturation		80°C	10 min.
	1Cycle	Annealing		25°C	10 min.
	1Cycle	Extension		37°C	50 min.

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

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

11.- Add 2µl of template (cDNA) into the PCR Premix tube to total volume as 13µl.

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

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

14.- 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	54°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 7 μ l of PCR product, 7 μ l of Positive control and 2 μ l of BrigTM 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 : 582 bp

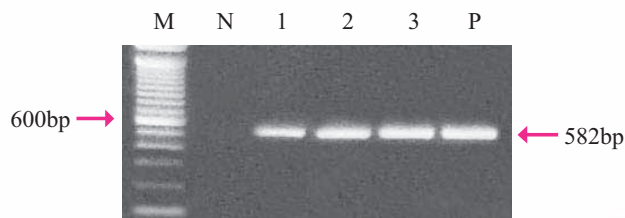


Fig 1. Electrophoresis of PCR product by VetPCRTM SIV Detection Kit
 Lane M : BrigTM Molecular Weight Marker (Bioingentech Ltd.)
 Lane N : Negative control
 Lane 1~3 : SIV Positive sample
 Lane P : Positive control

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 TM SIV Detection Kit 48	VET0004PR(48)
VetPCR TM SIV Detection Kit 96	VET0004PR(96)
Brig TM 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

Product use limitations warranty disclaimer

Bioingentech manufactures product for a number of Intended uses. Please refer to the product label for the Intended use statements for specific product. Bioingentech products contain chemicals which may be harmful if misused. Due care should be exercised with all Bioingentech products to prevent direct human contact. Each Bioingentech product is shipped with documentation stating specifications and other technical information. Bioingentech products are warranted to meet or exceed the stated specifications. Bioingentech's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Bioingentech make no other warranty of any whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTE WITH RESPECT TO BIOINGENTECH PRODUCTS. In no event shall Bioingentech be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Bioingentech products to perform in accordance with the stated specifications.

2004 – 2009 Bioingentech corporation. All rights reserved.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our web site for more information.

All prices and specifications are subject to change without prior notice

Product claims are subject to change. Please contact Bioingentech technical services or access the Bioingentech online catalog for the most up-to-date information on Bioingentech products.