VDx® SIV NF MP RT-PCR

Cat. No. NS-SIV-12

1. Description

VDx® SIV NF MP RT-PCR Kit is provides a range of testing for the detection of Swine Influenza Virus(SIV) by RT-PCR method. VDx® PCR Kit is formulated for the maximal stability of Taq polymerase(and Reverse Transcriptase) that has been dried together with reaction buffer, dNTP and stabilizer. It contains loading dyes for further convenience of use. Thus, the reaction mixtures after PCR cycles are ready for agarose gel electrophoresis. VDx® PCR Kit is ready-to-use PCR mixture containing concentration of components required for RT-PCR in one tube. For reactions, simply add template (RNA or DNA) and primer mix.

2. Storage

The components of VDx® SIV NF MP RT-PCR Kit should be stored at -20°C, under this condition, the kit is stable until expiration date stated on the label.

3. Contents

Reagents		96 Tests
1)	RT-PCR Premix(SIV NF)	50 T
2)	SIV NF primer mix	400㎕ X 2
3)	Control DNA	100㎕ X 1
4)	Instruction manual	1ea

4. Template preparation

- Test Sample : whole blood, serum, semen and tissue homogenates from pigs (The samples should be kept as fresh as possible and frozen during storage.).
- Template genes are extracted from 100~300㎕ of sample using QIAmp Viral RNA Mini Kit (Qiagen). Refer to the manufacturer's instructions for gene extraction methods.

* The gene extraction kit can be used with other products, but please check the manufacturer's manual in advance.

5. PCR method

❖ SIV NF MP RT-PCR (#NS-SIV-12)

1) Prepare appropriate PCR Premix tubes.
2) Add 15㎕ of primer mix solution into PCR premix tube.
3) Add 5㎕ of template RNA into the PCR premix tube.
4) Gently mixed and briefly centrifuged.
5) Perform PCR reaction of samples as the below process using PCR machine.

6. Detection of Amplified Products

1) Prepare 1.5% agarose gel containing Ethidium Bromide (Et-Br).
2) Load 5 ㎕ of PCR product on agarose gel without adding a loading dye buffer and perform electrophoresis.
3) Run electrophoresis by 100V(required about 20~40min).
4) Identify the result on ultra-violet(UV) transilluminator.

Step	RT-PCR Cycle(20㎕ reaction)		
	Temp.	Time	Cycle
cDNA synthesis	50℃	30 min	1 cycle
Initial inactivation	95℃	15 min	1 cycle
Denaturation	94℃	20 sec	40 cycles
Annealing	55℃	20 sec	
Extension	72℃	30 sec	
Final Extension	72℃	10 min	1 cycle

❖ Caution : Use of Control DNA

1) The control DNA contained in the product is designed to amplify the 575bp gene.
2) If a 575bp gene is detected in the sample, it can be judged that the Control DNA is contaminated.
3) Control DNA is used as a control reagent to check whether gene amplification occurs. It is added last using Filter tip to prevent contamination with sample.

VDx® SIV RT-PCR

Cat. No. NS-SIV-11

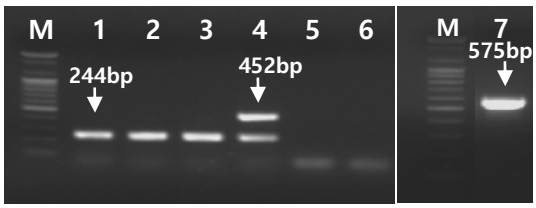
7. Interpretation

1) Control DNA can identify amplified products of 575 bp.
2) If the band of the below size is confirmed in the sample below, it is judged as the POS.

Virus	Target gene	Size
SIV common	M	244 bp
SIV Newflu	M	452 bp
Control DNA	-	575 bp

Gel Electrophoresis (1.5% Agarose)

SIV RT-PCR(NS-SIV-12)



M: Size Marker
Lane1: SIV (H1N1, 244bp)
Lane2: SIV (H1N2, 244bp)
Lane3: SIV (H3N2, 244bp)
Lane4: SIV (H1N1 Newflu, 244bp/452bp)
Lane5: NEG
Lane6: NEG
Lane7: Control DNA (575bp)

8. Notice

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. For in Vitro Use Only.
- Do not use any reagent after the expiration date.
- Do not use it with reagents of other products.

9. 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.

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