VDx® PRRSV ORF7 RT-PCR
VDx® PRRSV NA/EU Typing Nested PCR

Cat No. NS-PRR-11, NS-PRR-12

1. Description

VDx® PRRSV ORF7 RT-PCR / Nested PCR Kit is provides a range of testing for the detection of Porcine Reproductive and Respiratory Syndrome Virus(PRRSV) by RT-PCR (or 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® PRRSV ORF7 RT-PCR / Nested 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	ORF7 RT- PCR	Nested PCR
RT-PCR Premix(PRRSV)	96T	-
PCR Premix(PRRSV nested)	-	96T
Control DNA	100㎕ X 1	-
PRRSV Primer mix	800㎕ X 2	-
PRRSV nested Primer mix	-	800㎕ X 2
Instruction manual	1ea	1ea

4. Template preparation

4.1 Target Sample : whole blood, serum, plasma, semen and tissue(The samples should be kept as fresh as possible and frozen during storage.).
4.2 Extract genes from 100~300㎕ of sample using Qiagen Viral RNeasy Kit. 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

❖ PRRSV ORF7 RT-PCR (#NS-PRR-11)

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.

❖ PRRSV NA/EU Typing Nested PCR (#NS-PRR-12)

1) Prepare appropriate PCR Premix tubes.
2) Add 19㎕ of primer mix solution into PCR premix tube.
3) Add 1㎕ of template DNA(PCR products by PRRSV ORF7 RT-PCR) 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 (#NS-PRR-11)			Nested PCR (#NS-PRR-12)		
	Temp	Time	Cycle	Temp	Time	Cycle
cDNA synthesis	50℃	30 min	1 cycle			
Initial inactivation	95℃	15 min	1 cycle	94℃	3 min	1 cycle
Denaturation	94℃	20 sec	35 cycles	94℃	20 sec	25 Cycles
Annealing	55℃	30 Sec		55℃	20 sec	
Extension	72℃	40 sec		72℃	30 sec	
Final Extension	72℃	5 min	1 cycle	72℃	5 min	1 cycle

❖ Caution : Use of Control DNA

1) The control DNA contained in the product is designed to amplify the 756bp gene.
2) If a 756bp 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.

7. Interpretation

1) Control DNA can identify amplified products of 756 bp.
2) If the band of the below size is confirmed in the sample below, it is judged as the POS.

Product	Virus	Target gene	Size
PRRSV ORF7 RT-PCR	PRRSV NA type	ORF7	433 bp
	PRRSV EU type	ORF7	398 bp
PRRSV Nested PCR	PRRSV NA type	ORF7	287 bp
	PRRSV EU type	ORF7	184 bp

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.

Gel Electrophoresis (1.5% Agarose)

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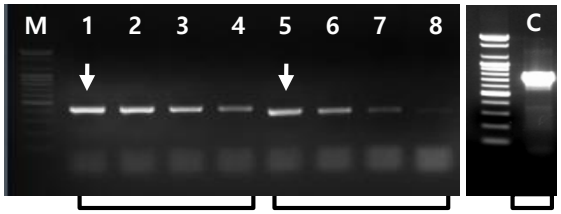

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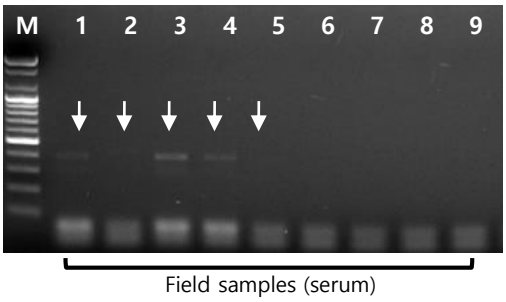
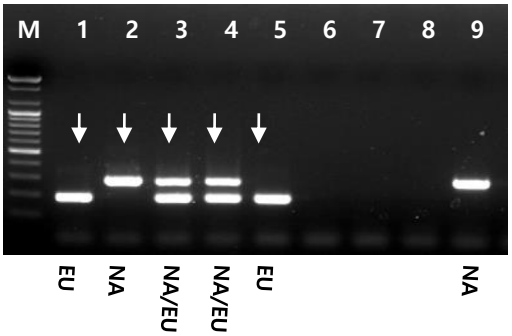


Nested PCR

PRRSV NA/EU typing Nested PCR(Cat#NS-PRR-12)



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