VDx® PCV2 ORF2 PCR

Cat No. NS-PCV-11

1. Description

VDx® PCV2 ORF2 PCR Kit is used for the detection of viral DNA of Porcine circovirus 2 (PCV2) by PCR method.

VDx® PCR Kit is formulated for the maximal stability of Taq polymerase 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 PCR in one tube. For reactions, simply add template (DNA) and primer mix.

2. Storage

The components of VDx® PCV2 ORF2 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)	PCR Premix(PCV2)	96T
2)	PCV2 primer mix	800㎕ 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 DNA 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

❖ PCV2 ORF2 PCR (#NS-PCV-11)

1) Prepare appropriate PCR Premix tubes.
2) Add 15㎕ of primer mix solution into PCR premix tube.
3) Add 5㎕ of template DNA into the PCR premix tube.
4) Gently mix and briefly centrifuge.
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	PCR Cycle (20㎕ reaction)		
	Temp	Time	Cycle
Initial inactivation	94℃	3 min	1 cycle
Denaturation	94℃	20 sec	35 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 317bp gene.
2) If a 317bp 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.

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7. Interpretation

1) Control DNA can identify amplified products of 317 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
PCV2	ORF2	493 bp
Control DNA	-	317 bp

Gel Electrophoresis (1.5% Agarose)

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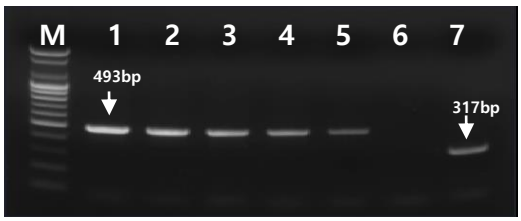

M: Size Marker
Lane 1: PCV2 (493bp)
Lane 2: PCV2 (493bp)
Lane 3: PCV2 (493bp)
Lane 4: PCV2 (493bp)
Lane 5: PCV2 (493bp)
Lane 6: Negative control
Lane 7: Control DNA(317bp)

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 difficulty to interpret results due to non-specific bands
   -> Reduce the 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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