VDx® Abortion MP PCR / RT-PCR II

CAT.NO. NS-ABO-11 / NS-ABO-12

1. Description

VDx® Abortion MP PCR/RT-PCR II kit is provides a variety of tests to detect four types of porcine abortion viruses (ADV, PPV, EMCV, JEV) 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® Abortion MP PCR/RT-PCR II kit should be stored at -20℃, under this condition, the kit is stable until expiration date stated on the label.

3. Contents

Reagents	ADV/PPV MP PCR	EMCV/JEV MP RT-PCR II
PCR Premix(ADV/PPV)	96T	-
RT-PCR Premix(EMCV/JEV)	-	96T
Control DNA	100㎕ X 1	100㎕ X 1
ADV/PPV Primer mix	800㎕ X 2	-
EMCV/JEV Primer mix	-	800㎕ X 2
Instruction manual	1ea	1ea

4. Template preparation

4.1 Target Sample : tonsil, lymph nodes, lung and Fetal pleural effusion(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

❖ ADV/PPV MP PCR(#NS-ABO-11)

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

❖ EMCV/JEV MP RT-PCR II(#NS-ABO-12)

1) Prepare appropriate PCR Premix tubes.
2) Add 15㎕ of primer mix solution into RT-PCR premix tube.
3) Add 5㎕ of template(sample) 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	ADV/PPV (#NS-ABO-11)			EMCV/JEV (#NS-ABO-12)		
	Temp	Time	Cycle	Temp	Time	Cycle
cDNA synthesis				50℃	30 min	1 cycle
Initial inactivation	94℃	3 min	1 cycle	95℃	15 min	1 cycle
Denaturation	94℃	45 sec	35 cycles	94℃	45 sec	35 Cycles
Annealing	55℃	30 Sec		55℃	30 sec	
Extension	72℃	45 sec		72℃	45 sec	
Final Extension	72℃	10 min	1 cycle	72℃	10 min	1 cycle

❖ Caution : Use of Control DNA

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) If the band of the below size is confirmed in the sample below, it is judged as the POS.

Product	Virus	Target gene	Size
ADV/PPV MP PCR	ADV	gD	282 bp
	PPV	VP2	458 bp
EMCV/JEV MP RT-PCR	EMCV	3D	286 bp
	JEV	E	480 bp

8. Notice

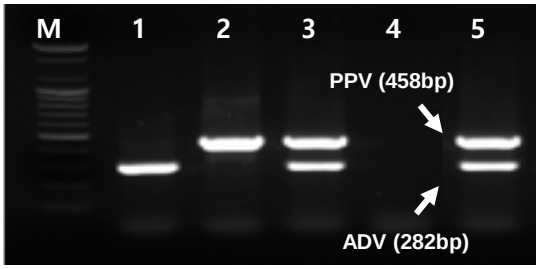
- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Do not use any reagent after the expiration date.
- Do not use it with reagents of other products.

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.

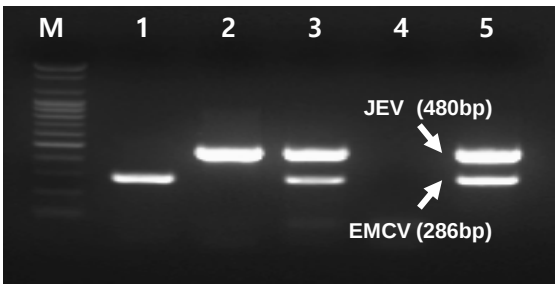
Gel Electrophoresis (1.5% Agarose)

Abortion MP PCR (PPV/ADV)



M: Size Marker,
Lane1: ADV sample(282bp)
Lane2: PPV sample(455bp)
Lane3: ADV,PPV mixing sample
Lane4: Negative control
Lane5: Control DNA

Abortion MP RT-PCR II (JEV/EMCV)



M: Size Marker
Lane1: EMCV sample(286bp)
Lane2: JEV sample(480bp)
Lane3: JEV, EMCV mixing sample
Lane4: Negative control
Lane5: Control DNA

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