

# VDx<sup>®</sup> Rotavirus VP6 RT-PCR

Cat. No. NM-ROT-11



## 1. Description

VDx<sup>®</sup> Rotavirus VP6 RT-PCR Kit provides a range of testing for the detection of Rotavirus A by RT-PCR method. VDx<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> Rotavirus VP6 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(Rota)	96T
2) Rota primer mix	800µl X 2
3) Control DNA	100µl X 1
4) Instruction manual	1ea

## 4. Template preparation

- Test Sample : stool and lesion tissue homogenates from pigs (The samples should be kept as fresh as possible and frozen during storage.).
- Template genes are extracted from 100~300µl 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

- ❖ Rotavirus VP6 RT-PCR (#NM-ROT-11)
  - 1) Prepare appropriate PCR Premix tubes.
  - 2) Add 15µl of primer mix solution into PCR premix tube.
  - 3) Add 5µl 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 µl 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(20µl reaction)		
	Temp.	Time	Cycle
cDNA synthesis	50°C	30 min	1 cycle
Initial inactivation	95°C	15 min	1 cycle
Denaturation	94°C	20 sec	40 cycles
Annealing	55°C	30 sec	
Extension	72°C	40 sec	
Final Extension	72°C	10 min	1 cycle

### ❖ Caution : Use of Control DNA

- 1) The control DNA contained in the product is designed to amplify the 644bp gene.
- 2) If a 644bp 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 644bp.
- 2) If the band of the below size is confirmed in the sample below, it is judged as the POS.

Virus	Target gene	Size
Rotavirus	VP6	332 bp
Control DNA	-	644 bp

## 8. Notice

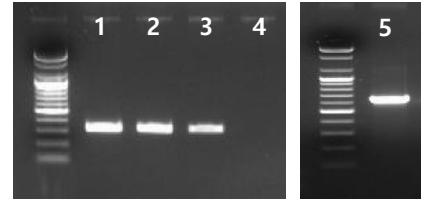
- For research purpose only. Not for use in diagnostic procedures for clinical purposes. For in Vitro Use Only.
- Do not use any reagent after the expiration date.
- Do not use it with reagents of other products.

## 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)

### Rotavirus VP6 RT-PCR (Cat# NM-ROT-11)



M: Size Marker

Lane 1: Rotavirus (332bp)

Lane 2: Rotavirus (332bp)

Lane 3: Rotavirus (332bp)

Lane 4 : Negative sample

Lane 5 : Control DNA

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