# VDx® BVDV qRT-PCR(T1/T2)

**Cat. No. NB-BVD-31**

## 1. Description

**VDx® BVDV qRT-PCR Kit** is used for the detection of viral RNA of Bovine Viral Diarrhea Virus (BVDV) by real-time PCR method.

This kit can measure the 5'UTR gene of BVDV quantitatively by using TapMan probe.

## 2. Contents

| No | Reagents | 96T |
|---|---|---|
| 1 | 2x qRT-PCR Master mix (A) | 1ml |
| 2 | 4x Oligo mix (B) | 500㎕ |
| 3 | Control DNA | 100㎕ |
| 4 | Manual | 1ea |

## 3. Storage

The components of VDx® BVDV qRT-PCR Kit should be stored at -20°C. The components of the kit are stable for 1 year from the manufacturing date (see expiry date on package).

## 4. Materials required (But not supplied)

- Dust-Free disposable gloves (without talcum powder).
- Microcentrifuge.
- Tube shaker.
- Real-time thermocycler.
- Micropipettes (10-100㎕).
- Sterile pipette tips (with filter).
- Sterile DNAses/RNAses free wster.

## 5. Precautions to avoid contamination

The following points should be read with care:

- Disposable items must be DNAse and RNAse-free.
- Use DNAse and RNAse-free autoclaved D.W. (25min, 120 ℃).
- Use sterile filtered tips.
- Maintain the qRT-PCR premix stored in ice during all the test procedure. Exposing them to temperatures above 4 ℃ reduces the efficacy of PCR.
- Repeated cycles of freezing and thawing may reduce the sensitivity of the reagents. Protect them from exposure to strong light until use.

To avoid contaminations leading to false positives, it is important to:

- Physically separate the positive PCR control from the remaining reagents of the kit.
- To handle another samples to be tested in a different place (or room) from the one where the amplified products are being analyzed.
- Add the positive PCR control in a different place/room from the one where the mix is added and where the samples to be tested are being handled.

## 6. Template preparation

- Test Sample : whole blood, 10% dilution feces and tissue homogenates from cattle (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.

## 7. Required equipment

Real-time thermal cycler capable of reading the following Fluorescence Dyes**: FAM, HEX(or VIC), Texas Red(or ROX)**

Examples of compatible thermal cycler : CFX96 Biorad , LC96 Roche, 7500 AB and Rotor-Gene Q Qiagen. Please contact us regarding suitability with other thermal cyclers

## 8. Preparation of the qRT-PCR

❖ Thaw the qRT-PCR Kit, ideally at 5℃(±3℃) in a refrige-rated rack. Thaw at room temperature 21℃(±5℃) only when the mix has to be used immediately after thawing.

1) Prepare and identify as many tubes for the amplification as samples to be processed, adding an additional tube for the positive amplification control, and another one for the negative control.

2) Take mixtures A and B out from the cooler keeping them in crushed ice. Make sure that they are homogenized well enough before taking out the required volume for the assay.

3) Prepare an suitable amount of amplification mixture for the number of samples to be processed. The volume of each reagent to be mixed for each of the samples is:

| | Per sample | For 10 samples |
|---|---|---|
| A : 2X Master Mix | 10㎕ | 100㎕ |
| B : 4X Oligo Mix | 5 ㎕ | 50 ㎕ |
| Final Master Mix | 15㎕ | 150㎕ |

The tube used for mixing should be kept in crushed ice during all procedures. Likewise, it is recommendable to prepare an excess amount of mixture in order to compensate for possible losses of volume during pipetting.

4) Once mixture is prepared, homogenise it well. Place the tubes previously labelled in crushed ice and add 15 µl of the mixture prepared in this way to each tube.

5) Add the following to the qPCR premix tube.
   - 5㎕ of Negative Control(DW)
   - 5㎕ of RNA extracted from each sample to be analyzed
   - 5㎕ of Control DNA(PC)

6) Cover the tubes with the caps.

7) Gently mixed and briefly centrifuged.

8) Perform PCR reaction of samples as the below process using PCR machine.

## 9. Programming the amplification

| No | Target | Fluorophore | Quencher |
|---|---|---|---|
| 1 | BVDV_Type1 | FAM | non-Fluorescent |
| 2 | BVDV_Type2 | Texas Red/ROX | non-Fluorescent |
| 3 | IPC | HEX / VIC | non-Fluorescent |

*For devices requiring an internal reference, use after setting non-mode.

| Step | qPCR Cycle (20㎕ reaction) | | |
|---|---|---|---|
| | Temp | Time | Cycle |
| cDNA synthesis | 50℃ | 30min | 1 cycle |
| Initial inactivation | 95℃ | 15min | 1 cycle |
| Denaturation | 95℃ | 10 sec | 40 cycles |
| Elongation | 60℃ | 60 sec | |
| hold | 4℃ | 10 sec | 1cycle |

*The fluorescence is read at the end of the elongation phase at 60 ℃.

## 10. Assay validation

The analysis of results is based on Cq (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).

The test is validated according to criteria outlined in the table below. Results should not be interpreted if any of these criteria are not met.

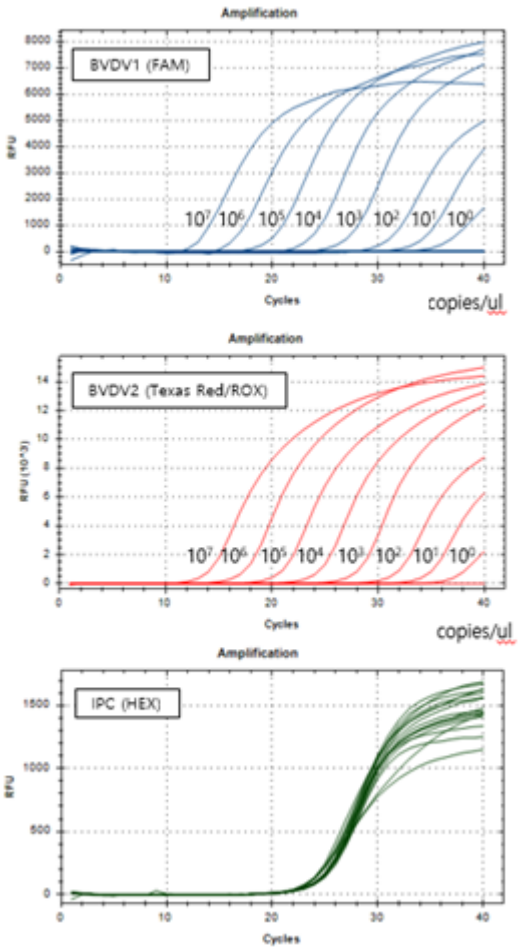
| | Expected result |
|---|---|
| Negative control | FAM : No detection if water used.<br>Texas red : No detection if water used.<br>HEX : Detection if virus-negative sample used |
| Control DNA | FAM & Texas red : detection |

## 11. Suggested interpretation of results

For each sample, results may be interpreted according to the following criteria.

| | BVDV | | IPC | Interpretation |
|---|---|---|---|---|
| | FAM | Texas red | HEX | |
| Case1 | POS | NEG | POS / NEG | Detected for BVDV_T1 |
| Case2 | NEG | POS | POS / NEG | Detected for BVDV_T2 |
| Case3 | POS | POS | POS / NEG | Detected for BVDV T1/T2 mix |
| Case4 | NEG | NEG | POS | Not Detected |
| Case5 | NEG | NEG | NEG | PCR reaction was inhibited |

## Technical data




**For questions or technical support,**
**Please contact : median@mediandx.com**