



## BVD RT-qPCR KIT

## User Guide

Catalog Number	Pack Size
ZT-BVD-01-100	100 Tests
ZT-BVD-01-500	500 Tests
ZT-BVD-01-1000	1000 Tests

### Content and Storage Condition

Content	Description	Quantity (20µL/Reaction)			Storage Condition	Routine Storage Condition
		100 Tests	500 Tests	1000 Tests		
SOFTEC Enzim Mix	Ready-to-use enzyme mixture for RT-qPCR	0,5 mL	2 x 1,25 mL	4 x 1,25 mL	-20°C	-20°C
SOFTEC Oligo Mix	Ready-to-use primer probe mixture for RT-qPCR	1,2 mL	5 x 1,2 mL	10 x 1,2 mL	-20°C	-20°C
SOFTEC PC	Positive Control	0.05 mL	0.05 mL	0.1 mL	-20°C	-20°C
SOFTEC NC	Negative Control	0.05 mL	0.05 mL	0.1 mL	-20°C	-20°C

### Features

- Kit content must be stored at -20°C until expiry date.
- Freeze thaw cycles for SOFTEC Enzyme Mix and SOFTEC Probe Mix are up to 5 times.
- To avoid repeated freezing and thawing as well as to minimize the contamination risk of stock solutions of reagents, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20°C.
- Kit is compatible with both qPCR cyclers that do not require and require an internal reference dye for normalization of fluorescent signal.

### Product Description

Bovine Viral Diarrhoea (BVD) and Mucosal Disease (MD) are caused by Bovine Viral Diarrhoea Virus (BVDV I, II), which is a single-stranded RNA virus belonging to the pestivirus genus, along with Classical Swine Fever Virus and Border Disease Virus in sheep. BVD is a significant infectious disease in cattle with economic implications. The severity of BVDV infections varies based on the immune status of the animals, causing gastrointestinal and respiratory symptoms of different degrees and reproductive problems. Fetal infection through the placenta can lead to abortions, congenital malformations, and persistently infected (PI or viraemic) calves if infected before immunocompetence. PI animals emerge only through prenatal infection, whereas postnatal infections result in transient viraemia and production of neutralizing antibodies. BVD RT-qPCR Kit comes in 4 different tubes and contains all the components necessary to perform RT-qPCR in a single tube on qPCR cyclers.



## SOFTEC BVD RT-qPCR Protocol

Clean the workbench to be worked with 0.5-1% (w/v) sodium hypochlorite first, then with 70% Ethyl alcohol. Thaw the reagents at 4°C on a cold shelf or on ice. If your time for work is limited, thaw them at room temperature. Mix each reagent several times by gently pipetting up and down, then centrifuge briefly. To avoid many freeze-thaw processes, please divide the mixes into sterile dnase/rnase free tubes at the first use. Prevent the fluorescent marked probes in the SOFTEC Probe Mix from being exposed to light and perform the operation in a dark environment every time you use it. Determine the total number of reactions per assay run. Each assay run should include the following:

- i. One Positive Control Sample that uses the PC (Positive Control) provided in the kit, as template.
- ii. One Negative Control Sample that uses the NC (Negative Control) provided in the kit, as template.
- iii. Total the number of Collected Samples

Component	Volume Per 20 µl Reaction		
	Positive Control	Negative Control	Test Sample
SOFTEC Enzyme Mix	5 µL	5 µL	5 µL
SOFTEC Oligo Mix	12 µL	12 µL	12 µL
Test Sample	-	-	3 µL
PC	3 µL	-	-
NC	-	3 µL	-

Mix the reaction mix thoroughly, then centrifuge briefly.

Run your RT-qPCR assay as shown in table below. NOTE: When programming your RT-qPCR run, choose the detection channel(s) as FAM and HEX.

Steps	Temperature	Time
Reverse transcription	50°C	10 min
Enzyme activation	95°C	5 min
X 45 Cycles	Denaturaton	95°C
	Annealing/Extension	60°C (READ)

Collect and analyze the data according to the instrument- specific instructions. Verify the amplification curve.

Target	Fluorophore/Channel
BVD target gene	FAM
Internal gene	HEX

### Data Analyses

Before starting the analysis, the threshold value for Bio-Rad brand devices, FAM and HEX channel should be set to 30 RFU.

	FAM	HEX	Interpretation
PC	Ct < 38	Ct < 38	Positive
NC	-	Ct < 38	Negative
Test Samples	Ct < 38	Ct < 38	Positive
	-	-	Invalid Test
	-	Ct < 38	Negative

## Safety and Hazards

### General Safety

Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

## General Requirements for Good Practices on PCR and RT-QPCR

### Laboratory Setup

To prevent contamination of the reaction mixture by previously amplified target sequences, it shall be ensured that separate work areas with their own apparatus are available. If possible, maintain separate work areas, dedicated equipment, and supplies for: Sample preparation, PCR setup, PCR amplification, Analysis of PCR products.

### Personnel

SOFTEC BVD RT-qPCR Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the molecular techniques and in vitro diagnostic procedures. Different sets of laboratory coats should be worn pre- and post-PCR. Disposable gloves should be worn at sample preparation and when setting up RT-qPCR.

### Protection of Product Performance and Analysis Efficiency

The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers. Master stock reagents should be kept on the cold block during the

PCR setup: if possible, the PCR setup should be performed on the cold block. Kit components should be mixed by gently shaking before use.

### Preventing Contamination

The kit should be stored away from nucleic acid sources and qPCR amplicons. The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate. Filtered and nuclease-free pipette tips should be used.

### Plate Layout Suggestions

In multi-targeted PCR runs, separate different targets by a row or by a column if enough space is available. If possible, put at least one well between unknown samples and controls.

### Warning

Do not preserve the product when the package is damaged.

## Assay Limitations

- SOFTEC BVD RT-qPCR Kit is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- A false negative result may occur if a specimen is improperly collected, transported, or handled.
- Detection of viral RNA may be affected by patient factors and/or stage of infection.

## Troubleshooting

Observation	Possible Cause	Recommended Action
Failure to detect FAM/HEX signals in Positive Control wells or HEX signals in Negative Control wells	PCR amplification failure	Make sure that the mix and PC/NC samples are pipetted correctly. Check that the thermal cycler settings and amplification program are correct. If there are no errors in them, renew the reagents and repeat the reaction. Contact with the manufacturer.
In the Negative Control wells, target-specific signals (FAM) are detected.	Contamination of the PCR	The cause of contamination may be due to hand errors made in sample processing, reagent contamination or environmental factors. Decontaminate the benchtop surfaces and other equipment where the PCR process is performed with 70% Ethyl alcohol and repeat the PCR process. Pipette Positive Control reactions last to avoid cross-contamination. Be sure to pay attention to the points in the section "General Requirements and Warnings on PCR and RT-qPCR Good Practices".
Detection of FAM signal in test sample wells, while HEX signal is not detected	A high copy number of target nucleic acid exists in samples, resulting in preferential amplification of the target-specific NA.	Repeat the PCR process for this sample. If the problem persists, dilute the sample with sterile nuclease-free water in a 1:1 ratio or SOFTEC NAT Solution that does not contain the patient sample and repeat the PCR process. If the problem persists, dilute the sample with sterile nuclease-free water in a 1:10 ratio or SOFTEC NAT Solution that does not contain the patient sample and repeat the PCR process. If all the results are the same, consider positive of the patient sample.
No signal is detected in any channel in the test sample wells	Inhibition Problem	Dilute the test sample in a ratio of 1:10 and repeat the PCR procedure. If the diluted sample does not show a positive result in the HEX channel, request a new sample from the patient. If the problem persists, contact with the manufacturer.