Instructions for use
InviScreen® IBDV Detection Kit

INVITEK diagnostics

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REF 6016001200 | Σ 100 tests | -25 °C / -15 °C | RUO

1. Intended use

InviScreen® Infectious Bursal Disease Virus (IBDV) Detection Kit is intended for the qualitative detection of IBDV RNA in samples of animal origin. This kit is intended for use by trained professionals in laboratory settings to aid in the diagnosis and surveillance of IBDV infections, the causative agent of Gumboro disease. It provides a reliable and sensitive method for identifying the presence of IBDV RNA in various animal samples, including epithelial swabs, tissues, and other relevant specimens. The results obtained from this kit should be interpreted in conjunction with clinical observations, history, and other laboratory tests to ensure accurate diagnosis and appropriate management of IBDV infections. Users should adhere to recommended biosafety protocols and handle all specimens, reagents, and materials following established laboratory practices to minimize the risk of contamination and ensure accurate results. Please consult and comply with local regulations, guidelines, and ethical considerations regarding the handling, testing, and reporting of infectious diseases in animals.

2. Product description

InviScreen® Infectious Bursal Disease Virus (IBDV) Detection Kit offers a reliable method for qualitatively determining the presence or absence of IBDV RNA in animal specimens by targeting the hypervariable region of VP2 through a real-time reverse transcription and multiplex amplification strategy. The method utilizes a hydrolysis probes, where amplification detection relies on the degradation of a hydrolysis probe during the PCR reaction, resulting in the emission of fluorescence. This enables the sensitive and specific detection of IBDV RNA in animal specimens, particularly tissues from Fabricius pouches obtained by scraping the internal epithelial layer using a sterile blade or swab. To ensure the accuracy of the results and to monitor potential PCR inhibitors, an Internal Amplification Control (IAC) detection primer/probe set is included in the kit. The IAC serves as a process control, allowing for the identification of samples that may contain substances inhibiting the PCR reaction.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D28.01	Primer/Probe Mix[1]	Targeted detection		1 tube, 250 µL
D28.02	RT-qPCR Master Mix[1]	Amplification		1 tube, 1250 µL
D28.03	Negative Control	Negative Control		1 tube, 1000 µL
D28.04	Positive Control	Positive Control		1 tube, 100 µL

[1] Reagents are supplied with a 5% of extra volume

4. Storage

Reagents should be stored sealed at -20 ± 5°C and may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles. Protect reagents from light exposure to prevent degradation.

5. Equipment and materials required (not provided)

- RNA extraction kit
- Real-Time PCR instrument
- Plates and/or tubes for qPCR
- 1.5 mL microcentrifuge tubes
- PCR cabinet
- Micropipettes (10, 200 and 1000 µL) and filter tips
- Vortex and microcentrifuge

6. Suitable test sample material

The test is applicable to biological samples of animal origin, specifically issues from the Fabricius pouch of birds collected by scraping the internal epithelial layer of the pouch with a sterile blade or swab.

7. Test Procedure

a. PCR Reaction Preparation

Allow all reagents to thaw at room temperature, vortex and spin briefly to avoid drops on the vial cap. For each RNA sample prepare a reaction mixture according to the table below:

REAGENT	VOLUME
RT-qPCR Master Mix	12.5 µL
Primer/Probe Mix	2.5 µL
RNAse/DNAse Free Water	5 µL
Total Volume	**20 µL**

1. Homogenize the reaction mixtures and pipette 20 µL into individual wells according to the predicted PCR plate set-up.
2. Add 5 µL of RNA template to each well.

At least one positive control reaction and one negative control reaction must be included in the PCR run, replacing the sample in these wells with 5 µL of Positive Control and 5 µL of Negative Control, respectively.

It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone. The addition of the positive control and sample RNA should preferably be carried out in a separate room.

b. Amplification Protocol

The amplification conditions are as follows:

	STEPS	TEMPERATURE	TIME	CYCLES
1	Reverse transcription	50 °C	15 min	1
2	Enzymatic Activation	95 °C	15 min	1
3	Denaturation	95 °C	15 sec	40
4	Hybridization/extension plate reading *	57 °C	60 sec	

* Fluorescence data must be obtained during this step through FAM and HEX channels.

c. Results Interpretation

The results should be interpreted in accordance with the analysis software recommended by the Real-Time PCR instrument manufacturer. The software monitors DNA amplification through the detection of fluorescence emitted by each probe, attributing a Ct value for each reporter dye found in each individual sample. Target DNA amplification is monitored in FAM (IBDV) and HEX (IAC) channels. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred bellow.

A result is considered positive when Ct ≤ 40

RESULT	Target DNA(FAM)	IAC (HEX)
Positive	+	+/-
Negative	-	+
Inconclusive [2,3]	-/?	-

[2] PCR inhibitions may be due to the presence of excessive DNA and/or PCR inhibitors. It is recommended to dilute the RNA extracted from the sample 1:10 or 1:100 in DNAse/RNAse free water and repeat the Real-Time PCR reaction. When applicable, the LOD of the method should be adjusted in accordance with the dilution factor.
[3] The appearance and characteristics of the amplification curves should be thoroughly considered. Incomplete amplification curves often denote low amount of RNA template. In this case, the positivity of the result is dubious, and the Real-Time PCR reaction should be repeated using a superior amount of RNA template.

8. Quality Control

The test can only be considered valid under the following control conditions:

CONTROLS	Target DNA	IAC
Positive Control	+	+
Negative Control	-	+

If no amplification is observed for the positive control, the test results are invalid and must be repeated. The positive control template is expected to amplify before Ct 25. If amplification is observed for the negative control, it indicates that the reagents have become contaminated while setting up the run, invalidating test results.

9. Performance Characteristics

Specificity: InviScreen® (IBDV) Detection Kit was designed to specifically detect IBDV RNA. The specificity was evaluated by analysing RNA extracted from various IBDV strains (IBDV strain V877; strain Cu-1M, strain V217, strain 2512, strain GM97, strain CH/80, strain 228E and strain D78) and cross reactivity tested using a large panel of infectious disease agents that include avian viruses, porcine viruses and bacteria.

Reference material	Results	
	Target (IBDV)	IAC
Infectious Bursal disease Virus (strain V877)	Positive	Positive
Infectious Bursal disease Virus (strain Cu-1M)	Positive	Positive
Infectious Bursal disease Virus (strain V217)	Positive	Positive
Infectious Bursal disease Virus (strain 2512)	Positive	Positive
Infectious Bursal disease Virus (strain GM97)	Positive	Positive
Infectious Bursal disease Virus (strain CH/80)	Positive	Positive
Infectious Bursal disease Virus (strain 228E)	Positive	Positive
Infectious Bursal disease Virus (strain D78)	Positive	Positive
Pseudomonas aeruginosa	Negative	Positive
Enterococcus faecalis	Negative	Positive
Salmonella enteritidis	Negative	Positive
Mycoplasma synoviae (MS)	Negative	Positive
Mycolasma gallisepticum (MG)	Negative	Positive
Porcine Respiratory & Reproductive Syndrome Virus (PRRSV)	Negative	Positive
Aujeszky Disease Virus (ADV)	Negative	Positive
Newcastle Disease Virus (NDV)	Negative	Positive
Avian Encephalomyelitis Virus (AEV)	Negative	Positive
Infectious Bronchitis Virus (IBV)	Negative	Positive

Detection Limit and sensitivity: The limit of detection (LOD) is often matrix dependent, and the sensitivity of the analysis may be reduced depending on the total RNA extracted from the actual ingredient in test, but also its quality. This way, the LOD needs to be determined through in-house validation.
The LOD was determined using positive reference material (DNA plasmid containing the gene of interest) produced in-house. Successive dilutions (base 10) were used, so that at the lowest dilution (highest concentration, $1x10^7$ copies/µL) all results are positive (for target gene) and at the last dilution (lowest concentration, 10 copies/µL) it is expected that all or most of the results will be negative. Under optimal conditions, the lowest amount of IBDV detected in 100% of the experiments was 10^2 viral copies/ µL.