Kylt®

Kylt® IBDV genogroup A1 (classical)

Real-Time RT-PCR Detection

www.kylt.eu
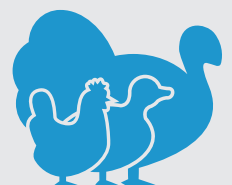
Kylt® IBDV genogroup A1 (classical)
Real-Time RT-PCR Detection

A. General

- Kylt® IBDV genogroup A1 (classical) kits are intended for the specific detection of viral RNA of *Infectious Bursal Disease Virus* (*IBDV*) genogroup A1 (classical) in samples positively tested for *IBDV* with Kylt® IBDV Screening (Art. 31044/31045). In conjunction with the vaccination scheme of the tested animals, the clinic present and the detection of the vaccines mentioned on page 8, conclusions can be drawn about the identity of the *IBDV* strain detected in the screening PCR.

- The kits are suitable for the analysis of samples from birds tissues and organs (e.g. Bursa of Fabricii, spleen, lymphoid tissue), swab samples of the aformentioned tissues and organs and samples from cultural processes with the aforementioned sample material.

- The qualitative testing with Kylt® IBDV genogroup A1 (classical) kits is based on a triplex Real-Time RT-PCR: In one reaction setting, the RNA target sequences for *IBDV* genogroup A1 as well as for the endogenous control (beta-Actin) are reverse transcribed (Reverse Transcription (RT)) and amplified in parallel with respective primer pairs in the Polymerase Chain Reaction (PCR). Amplified target gene fragments are detected via fluorescently labeled probes during the PCR reaction in real-time (Real-Time PCR). The probes specific for detection of amplified target genes from *IBDV* genogroup A1 and the endogenous control are labeled with fluorescent dyes FAM, Cy5 and HEX, respectively, and their emitted fluorescence is separately optically measured by the Real-Time PCR thermal cycler. By means of both individual analyses in one reaction vessel per sample and the Negative Control and Positive Control per run the *IBDV* genogroup A1-specific status of a sample can be evaluated in the end. This way, results can be achieved within a few hours after sample receipt.

- These kits were developed for use by trained laboratory personnel following standardized procedures. This Direction For Use must be followed strictly.

B. Reagents and Materials

- Kylt® IBDV genogroup A1 (classical) RT-qPCR kits are available and comprise the following reagents:

Reagent	Colour of Lid	100 Reactions Article No 31667	25 Reactions Article No 31668	Store at
2x RT-qPCR-Mix	○ transparent	4 x 280 µl	1 x 280 µl	≤ -18 °C
Detection-Mix	● green	4 x lyophilizate (final 150 µl each)	1 x lyophilizate (final 150 µl each)	≤ -18 °C
Positive Control	● red	4 x lyophilizate (final 50 µl each)	2 x lyophilizate (final 50 µl each)	≤ -18 °C
Negative Control	● blue	1 x 1 ml	1 x 1 ml	≤ -18 °C

- After receipt, the components are immediately stored at ≤ -18 °C. Avoid repeated freezing and thawing of all the reagents and keep them thawed as short as possible. If occasional processing of few samples only is expected you may prepare appropriate aliquots of reagents before storage at ≤ -18 °C. Prepare aliquots in such a way that freeze-thaw-cycles are reduced to a maximum of three. The Negative Control can alternatively be stored at +2°C to +8°C.

- The components are to be used within the indicated shelf life (see box label). The components of different batches may not be mixed.

- The Detection-Mix needs to be stored protected from abundant light. Do not expose to direct (sun)light. Before first use, rehydrate the lyophilized Detection-Mix: add 150 µl of the Negative Control per vial of Detection-Mix, briefly incubate at room temperature and mix by pulse-vortexing. Generate aliquots of suitable volumes and store them at ≤ -18 °C.

- Before its first use, rehydrate the Positive Control: add 50 µl of Negative Control per vial, briefly incubate at room temperature and mix thoroughly by repeated vortexing. It is recommended to generate aliquots of suitable volumes and store them at ≤ -18 °C.

C. Equipment and Reagents not included

- This detection method can be used on all commercially available Real-Time PCR thermal cyclers that detect the emitted fluorescence of the fluorescent dyes FAM and HEX and Cy5 (emission 520, 550 and 670 nm, respectively). Note that default normalization option against ROX (e.g. using ABI cyclers) must be deactivated.

- Apart from the disposables, the following further devices are needed and are not included in the Kylt® IBDV genogroup A1 (classical) kits:
  - RNA preparation kit / protocol (e.g. Kylt® RNA / DNA Purification products)
  - Table top microcentrifuge
  - Vortex
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge for PCR tubes or plates
  - Real-Time PCR Thermocycler

- Accessory Kylt® products: see chapter F "Related and Accessory Products".

- We recommend the exclusive use of certified Nuclease-free disposables as well as powder-free protective gloves. Please wear gloves during the entire experimental procedure. Gloves need to be changed frequently, especially after spillage or suspected contaminations.

D. Control Reactions

- The <u>Positive Control</u> allows for control of the specificity and efficiency of the reagents and the reaction itself, including the performance of RT and Real-Time PCR and of the Real-Time PCR thermal cycler.

- The <u>Negative Control</u> allows for exclusion of contaminations. The sample testing is only valid if both, Positive and Negative Controls, are used and verified for validity in every Real-Time PCR run.

- The <u>Internal Control</u> is based on <u>detection of beta-Actin RNA</u>, which is ubiquitous in the cells of the host that the sample is derived from. The beta-Actin RNA/endogenous RNA target gene is co-amplified (channel HEX) with every single reaction and allows for evaluation of sufficient sampling, sample storage and shipment, sample preparation and the Real-Time RT-PCR run itself.

- It is recommended to run one or more of a <u>RNA Isolation Control (RIC)</u> per set of RNA preparation, depending on the total number of samples processed at once. The RIC is a "mock sample" composed of the plain sterile buffer used for raw sample processing. It is randomly placed between the samples, processed like a normal sample and allows to detect potential contaminations of the reagents used (additionally to the Negative Control reaction) as well as for the detection of potential carryover contaminations between individual samples, e.g. during the RNA preparation process.

E. Protocol *(see also „Protocol At A Glance" at the end of this Direction For Use)*

- The overall protocol of the analysis consists of the following main workflow:

  1. Sample Preparation
  2. RNA Preparation
  3. Reaction Setup, Reverse Transcription and Amplification (Real-Time RT-PCR)
  4. Data Analysis – Validity and Qualitative Result

- We recommend proceeding through the protocol without interruption to avoid potential degradation of the processed samples and reagents. If necessary, you may store the final RNA preparation at ≤ -18 °C or ≤ -70°C until further processing. Avoid repeated freezing and thawing of the RNA preparations.

1. Sample Preparation

- We recommend <u>pooling</u> of at most five samples or samples from five individuals, respectively, per RNA preparation.

- Pool <u>swabs</u> in a sufficient volume of sterile buffer (e.g. 1 ml of Normal Saline or 0.1 x TE), let the swabs soak for an adequate period of time and finally wash out the swabs by thorough pulse-vortexing. The washed out supernatant is used for RNA preparation.

- <u>Tissue and organ</u> samples are homogenized thoroughly in sterile buffer (see above) and a suitable volume is used for the RNA preparation.

- Material derived from cultural processes, i.e. cell culture supernatant or allantoic fluid, can be used directly for RNA preparation.

2. RNA Preparation

a) Kylt® RNA Preparation (requires Kylt® RNA / DNA Purification products (available separately))

- For detailed information on the RNA preparation process please refer to the manual of Kylt® RNA / DNA Purification products.

b) RNA Preparation by other Methods

- Other kits or in-house methods to purify RNA may be used, as long as the quality leads to satisfactory amplification and detection of the Internal Control RNA.

3. Reaction Setup and Amplification (Real-Time RT-PCR)

- Before each use, briefly vortex and spin down the 2x RT-qPCR-Mix, rehydrated Detection-Mix and Negative Control.

- To determine the total number of reactions needed, count the number of samples and add two more for the Negative Control and the Positive Control (and RIC(s), if processed).

- The master mix is prepared for the appropriate number of reactions using the components listed below. Vortex, spin down and add 16 µl of the finalized Master-Mix to each of the PCR tubes or plate wells ("cavities").

Reagent	Volume (µl)	
	per Reaction	e.g. n=7
2x RT-qPCR-Mix	10 µl	70 µl
Detection-Mix	6 µl	42 µl
Total Master-Mix	**16 µl**	**112 µl** dispense 16 µl per reaction
RNA (Negative Control / sample RNA / RIC(s) / Positive Control)	4.0 µl	
Total Reaction	**20.0 µl**	

- A larger volume of a ready to use Master-Mix can be prepared and stored at ≤ -18 °C for convenient use over a longer period of time up to the expiry date given on the label. In case of frozen storage the Master-Mix should be aliquoted in such a way that freeze-thaw-cycles are reduced to a maximum of three..

- Keep exposure of the 2x RT-qPCR-Mix, Detection-Mix and prepared Master-Mix to (sun)light as short as possible and return it back to appropriate storage temperature right after application. Avoid the formation of bubbles when pipetting the Master-Mix, samples and controls.

- Add 4 µl of the Negative Control to the corresponding cavity and seal it individually, if possible.

- Add 4 µl of each RNA preparation (including RIC(s), if processed) to the corresponding cavities and seal them individually, if possible.

- To minimize risk of potential cross-contaminations, 4 µl of the Positive Control are added to the corresponding cavity after all previous samples and control reactions are set up. Before each use, briefly vortex and spin down the rehydrated Positive Control (see also chapter B "Reagents and Materials").

- If not already done, finally seal the cavities. It is recommended to briefly spin them down before the start of the Real-Time PCR run.

■ Place the cavities in the Real-Time PCR thermal cycler and run the test with Kylt® Profile I as given below.

Kylt® Profile I			
Step No	Description	Temperature	Duration	
1	Reverse Transcription	50 °C	10 min	
2	Activation of Polymerase	95 °C	1 min	
3	Denaturation	95 °C	10 sec	
4	Annealing & Extension	60 °C	1 min	42 cycles
5	Fluorescence Detection	channels FAM, HEX and Cy5		

■ Kylt® Profile I allows for combined run of this and most other Kylt® RT-qPCR detection methods as well as Kylt® PCR detection products.

■ In the event of a combined Real-Time RT-PCR run, make sure all necessary channels are detected.

■ Please follow the specified instructions of your Real-Time PCR thermal cycler as recommended by the manufacturer.

4. Data Analysis – Validity and Qualitative Result

General

■ The amplification data can be processed automatically using the specific software tool of your Real-Time PCR thermal cycler. Alternatively, the threshold can be set manually considering the following directions: The threshold should cross the FAM-, Cy5- and the HEX-curves in the linear increase of their slope (log scaling of the y-axis). By setting the threshold, the crossing points with the FAM-, Cy5- and HEX-curves determine the respective cycle threshold (Ct), which is negatively correlated with the initial concentration of copies of the target genes in the Real-Time RT-PCR reaction.

■ Only curves with the typical exponential amplification, meaning the curve of the raw data shows a flat baseline at the beginning, followed by a clear (exponential) slope in fluorescence and possibly reaching a plateau-phase (y-axis in log scaling), should be regarded as positive.

■ The actual test analysis starts with the validity check of the entire Real-Time RT-PCR run. Afterwards, by means of the Internal Control the validity of each sample reaction and its true test result can be verified according to the Ct-value of the Internal Control channel (HEX). Finally, the *IBDV* genogroup A1-specific status of each sample is analyzed (FAM and Cy5).

Test Evaluation - Control Reactions

- The **Real-Time PCR test run** is only **valid** if the curves of the control reactions can be evaluated as follows:

Control Reactions	Channel		
	HEX	FAM	Cy5
Negative Control	negative	negative	negative
Positive Control	**positive**	**positive**	**positive**

- For a valid test the FAM- and Cy5-Ct-values of the Positive Control have to be > 15 and ≤ 35 and the HEX-Ct-value has to be ≤ 35.

- The HEX-, FAM- and Cy5-Ct-values of the Negative Control have to be negative (Ct > 35).

- Depending on the cycler and consumables used strong signals in the FAM-channel of the Positive Control may lead to weak background signals in the HEX-channel.

- If one or more of a RNA Isolation Control (RIC(s)) is processed, its FAM-, Cy5- and HEX-curve must be negative (Ct > 35 for HEX-curves).

- *IBDV* genogroup A1 (classical) strains can only be analyzed in *IBDV* positive samples.

Test Evaluation - Samples

Target	Channel	Signal				
Internal Control	HEX	**positive**	positive / negative	positive / negative	positive / negative	negative
IBDV genogroup A1 (classical)	Cy5	negative	**positive**	negative	**positive**	negative
	FAM	negative	negative	**positive**	**positive**	negative
The sample is *IBDV* genogroup A1 (classical)		**negative**	**negative**	**negative**	**positive**	**inhibited**

- A **sample** is **negative for *IBDV* genogroup A1 (classical)**, if its HEX-curve is positive (Ct ≤ 35), but its FAM- and Cy5-curve are negative.

- A **sample** is also **negative for *IBDV* genogroup A1 (classical)**, if only the FAM- or Cy5-curve is positive, independent of the HEX-curve.

- A **sample** is **only positive for *IBDV* genogroup A1 (classical)**, if its FAM- and Cy5-curves are positive (Ct ≤ 42), independent of the HEX-curve.

- A **sample** is **inhibited** if neither the FAM-, Cy5- nor the HEX-curve are positive.

- **Recommendation:** In the case of an inhibited sample you may repeat the test by using e.g. 1:4 dilution of the respective RNA preparation. The Negative Control is used as the diluting agent. Preferably, repeat the RNA preparation procedure from the start using less or alternative raw sample material. Afterwards, you may additionally conduct an ethanol precipitation to concentrate the RNA.

- Convenient and reliable sample data entry, Real-Time PCR start, final qualitative analysis and documentation can be conducted with the Kylt® Software, please inquire.

■ Kylt® IBDV genogroup A1 (classical) also detects the following vaccines:

Vaccine	Genogroup
Avipro® IBD Xtreme	A1a
AviPro® Gumboro Vac	A1b
Avipro® Precise	A1b
Avishield IBD INT	A1b
Avishield IBD Plus	A1b
BUR 706	A1b
CEVAC® IBD L	A1a
CEVAC® TRANSMUNE IBD	A1a
Gallivac IBD	A1b
Innovax®-ND-IBD	A1a
HIPRAGUMBORO® CH/80 - CW	A1b
HIPRAGUMBORO® GM97 - G97	A1b
Nobilis® Gumboro D78	A1b
Nobilis® Gumboro 228E	A1b
Novamune®	A1b
Poulvac® Bursaplex®	A1a
Poulvac® Bursine 2	A1a
Vaxxitek™ HVT + IBD	A1a

F. Related and Accessory Products

Product	Article No	Content	Description
Kylt® IBDV Screening	31044 / 31045	100 / 25	PCR Kit for the specific detection of viral RNA of *Infectious Bursal Disease Virus* (*IBDV*).
Kylt® RNA / DNA Purification	31315	50	Combined RNA and DNA purification from veterinary samples (spin-column based).
Kylt® RNA / DNA Purification HTP	31826	4 x 96	Magnetic bead based combined RNA and DNA purification kit for veterinary diagnostic samples. Suitable for Kylt® Purifier and Kylt® Purifier 48.
Kylt® Purifier	31436	1 unit	Purification system for magnetic bead based kits. Up to 96 samples are processed in under 30 minutes. Intended for high-throughput laboratories.
Kylt® Purifier 48	31436	1 unit	Purification system for magnetic bead based kits. Up to 48 samples are processed in under 30 minutes. Intended for low to medium throughput laboratories.
Kylt® Purifier Spin Tips	31434	5 Sets	Plate with 96 separate spin tips, used by the Kylt® Purifer to mix the well contents by stirring. Sufficient for 480 samples.
Kylt® Purifier Plates	31435	20 Plates	Plates to be used for the several reactions and reagents during automated nucleic acid purification. Sufficient for 320 to 480 samples (depending on device and protocol).

G. Ordering information

For a fast and efficient service please send your order to orders@kylt.eu and please provide the following information:

- Delivery address

- Invoice address

- Purchaser contact telephone number

- End user name and telephone number (if different)

- Purchase order number, product name and cataloge number

- Quantity and size of products

- Indicate if your account is VAT exempt

Production:

AniCon Labor GmbH | Muehlenstr. 13 | D-49685 Hoeltinghausen | Germany | www.kylt.eu | info@kylt.eu

Development, manufacturing and distribution of Kylt® *In-Vitro* Diagnostica is certified according to ISO 9001:2015.

Kylt® is a registered trademark.

For veterinary use only. For *in vitro* use only. Regulatory requirements vary by country, not all of the products described herein may be available in your geographic area.

© 2023 AniCon Labor GmbH. All rights reserved. The trademark mentioned herein is the property of AniCon Labor GmbH or their respective owners.
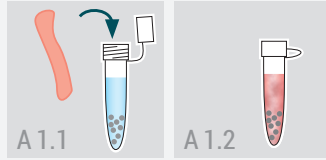
Management System
ISO 9001:2015

TÜVRheinland®
ZERTIFIZIERT
www.tuv.com
ID 9000003434

PROTOCOL AT A GLANCE
Real-Time RT-PCR Setup

1

A Organs and tissue samples,
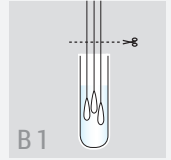e.g. Bursa of Fabricii, spleen, lymphoid tissue

1. Sample Preparation

1.1 transfer tissue in a tube of normal saline (0,9%) or 0,1 x TE

1.2 homogenization

A 1.1 A 1.2

B Other samples,
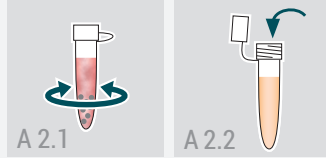e.g. swab samples, cultural material

1. Sample Preparation

pooling of max. permissible number of samples in a tube of normal saline (0,9%) or 0,1 x TE
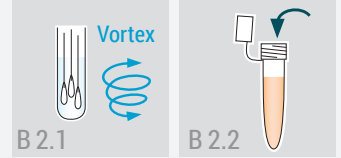
B 1

2

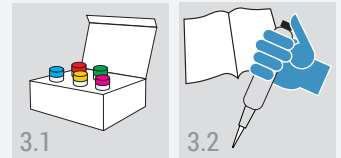### 2. Centrifugation

2.1 centrifuge

2.2 transfer supernatant

A 2.1 A 2.2

2. Washout

2.1 briefly vortex
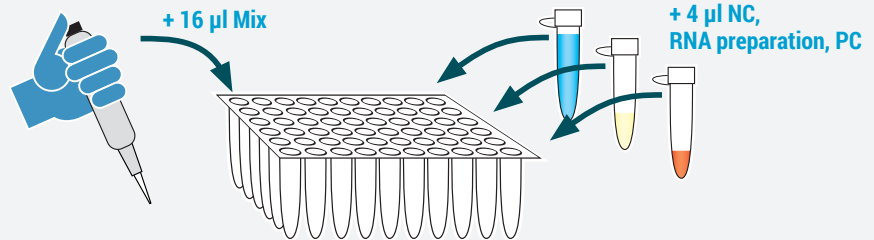
2.2 transfer supernatant

Vortex

B 2.1 B 2.2

3

3. RNA Preparation

3.1 use of a commercially available RNA preparation kit

3.2 further preparation according to the instructions of the corresponding kit

3.1 3.2

4

Dispense Master-Mix and add 2 µl NC, RNA preparation, PC

+ 16 µl Mix

+ 4 µl NC, RNA preparation, PC

5

Seal cavities, spin down (recommended), and start cycler

6

Analysis

normalized fluorescence

positive sample

CT

exponential phase

threshold

baseline

negative sample

cycle

Kylt®