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Kylt® Duck Enteritis Virus

PCR Detection Kit for Duck Enteritis Virus

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100 / 25 reactions
in vitro Diagnosticum for birds

A. Introduction

- Kylt® Duck Enteritis Virus PCR Detection Kit is for detection of Duck Enteritis Virus in samples from birds, such as tissues and organs (e.g. liver, pancreas, kidney, spleen, cecal tonsils, intestinal mucosa), swab samples of the aforesaid tissues and organs, feces and sampling material derived from cultural processes with the aforementioned samples (e.g. culture harvest).

- Kylt® Duck Enteritis Virus comprises all reagents and controls for differentiation of viral DNA of Duck Enteritis Virus (DEV). The qualitative testing of Kylt® Duck Enteritis Virus is based on the Polymerase Chain Reaction (PCR). By using DEV-specific oligonucleotides, the target gene of interest in a sample is specifically amplified. Following amplification the PCR reaction is analyzed by agarose gel electrophoresis for qualitative test result. By analyzing the detection of the target gene in the sample, in the Negative Control and in the Positive Control the DEV-specific status of the sample can be evaluated in the end. This way, results can be achieved within few hours after sample receipt.

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

B. Reagents and Materials

- Kylt® Duck Enteritis Virus contains the following reagents:

Reagent	Color code of lid	Quantity in kit with 25 / 100 reactions	Storage conditions
2x PCR-Mix	○ white	1 x / 4 x 280 µl	-18 °C to -20 °C
10x Loading Dye	○ transparent	1 x / 4 x 60 µl	-18 °C to -20 °C
Primer-Mix	● brown	1 x / 4 x lyophilisate (final 160 µl each)	-18 °C to -20 °C
Positive Control	● red	2 x / 4 x lyophilisate (final 20 µl each)	-18 °C to -20 °C
Negative Control	● blue	1 x 1 ml	-18 °C to -20 °C

- After receipt, the kit and its components are immediately stored at -18 °C to -20 °C. Avoid repeated freezing and thawing of the kit or its components and keep them thawed as short as possible. If needed, generate appropriate aliquots upon its first thawing. The kit and its components are to be used within the indicated shelf life (see label on the outer packing), if stored properly. The components of different batches may not be mixed.
- Before first use, rehydrate the lyophilized Positive Control: add 20 µl of Negative Control (Nuclease-free water) per vial, incubate briefly at room temperature and mix thoroughly by pulse-vortexing. Generate aliquots of suitable volumes and store them at -18 °C to -20 °C.
- Before first application, the lyophilized Primer-Mix is rehydrated: add 160 µl of Negative Control (Nuclease-free water) per vial, briefly incubate at room temperature and mix by repeated vortexing. Generate aliquots of suitable volumes and store them at -18 °C to -20 °C.
- This kit can be used on all commercially available PCR thermal cyclers.
- We recommend to exclusively use certified Nuclease-free materials and powder-free protective gloves. Please wear gloves during the entire experimental procedure. Gloves have to be changed frequently, especially after spillage or other suspected contamination. Pipette tips must be changed between each sample to avoid cross-contamination.

- Apart from the disposables, the following devices are needed (not included in this kit):
  - DNA extraction kit /protocol
  - Table top microcentrifuge
  - Vortex
  - Micropipettes covering volumes of 1 µl to 1000 µl
  - Centrifuge for PCR tubes
  - PCR thermal cycler
  - Equipment, media and disposables for agarose gel electrophoresis

C. Control Reactions

- The Negative Control included in this kit provides the ability to exclude possible contaminations of the reagents. The Positive Control included in the kit provides the ability to monitor the specificity and efficiency of the reagents used, i.e. the performance of PCR itself and of the PCR thermal cycler, respectively. The sample testing is only valid if both the Positive Control and the Negative Control reactions are included in every PCR run and used to verify the validity of such run.

D. Protocol

- The overall protocol of the DEV analysis consists of the following workflow:
  1. sample processing
  2. DNA preparation
  3. reaction setup & amplification (PCR)
  4. agarose gel electrophoresis
  5. 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 DNA preparation at -18 °C to -20 °C or -70 °C to -80 °C until further processing of the DEV detection, respectively. Avoid repeated freezing and thawing of the DNA preparations.

1. Sample Processing

- We recommend pooling at most five individual samples or samples of five individuals, respectively, per DNA preparation.
- Swabs are pooled in a sufficient volume of sterile buffer (e.g. Normal Saline or 0.1 x TE) and soaked for an adequate period of time. Then, the sample is washed out thoroughly by pulse-vortexing and the washed out supernatant is used for DNA preparation. Single small swabs may directly be immersed in lysis buffer.
- Tissue samples are homogenized thoroughly in sterile buffer (see above) and a suitable volume is administered to DNA preparation.
- Cultural material is directly processed with an appropriate DNA preparation protocol.

2. DNA Preparation

- For detailed information on the DNA preparation process, please refer to the Direction For Use or Standard Operating Procedure of the respective kit or in-house method, respectively.

3. Reaction Setup and Amplification

- Before each use, briefly vortex and spin down the 2x PCR-Mix, the 10x Loading dye and the rehydrated Primer-Mix (see also chapter B "Reagents and Materials").
- Determine the number of reactions: the number of your samples plus Positive Control and Negative Control.
- Prepare a Master-Mix containing the 2x PCR-Mix, 10x Loading Dye and the Primer-Mix for the appropriate number of reactions. Then add 18 µl of the Master-Mix to each of the PCR tube or well of plate ("cavity"). The PCR is set up in the given order:

Reagent	Volume (µl)	
	Per reaction	e.g. n=7
2x PCR-Mix	10.0	70.0
10x Loading Dye	2.0	14.0
Primer-Mix	6.0	42.0
Total Master-Mix	**18.0**	**126, dispense 18 µl per reaction**
Template (Negative Control / DNA preparation / Positive Control)	2.0	
Total Reaction	**20.0**	

- Keep the 2x PCR-Mix, the 10x Loading Dye and the Primer-Mix thawed as short as possible (preferably on cooling block or in ice water) and return all back to -18 °C to -20 °C right after application. Avoid the formation of bubbles when pipetting the Master-Mix, samples and controls.
- Add 2 µl of Negative Control to the corresponding cavity and seal it.
- Add 2 µl of sample DNA (final DNA preparation) to the corresponding cavity and seal it.
- To minimize risk of potential cross-contaminations, 2 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").
- It is recommended to spin the cavities down before start of the PCR run.

- Place cavities in the PCR thermal cycler and run the test using the following parameters:

Step	Temperature	Duration	
Activation of Polymerase	94 °C	3 min	
Denaturation	94 °C	30 sec	35 cycles
Annealing	57 °C	30 sec	
Extension	72 °C	60 sec	
Post-PCR cooling (optionally)	7 °C	hold	

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

4. Agarose Gel Electrophoresis

General

- The expected DEV-product size is 210 bp, you may run any standard agarose gel electrophoresis method appropriate for this product size. An appropriate method is described briefly below.
- Prepare a 2% standard agarose gel for separation of the DNA sample after PCR amplification.
- The PCR reactions already contain loading buffer including electrophoresis tracking dyes that migrate at approximately 4 kbp and 50 bp and are ready for loading on agarose gel following PCR.
- Load the wells of agarose gel electrophoresis with 5 µl of the PCR reactions from the DNA sample(s), Positive Control and Negative Control, respectively. Load at least an additional well of agarose gel with 2 µl of e.g. an 100 bp reference DNA ladder. Make notes of the position of sample(s), controls and ladder.
- Run the electrophoresis at a voltage of approximately 15 V/cm (the distance in cm refers to the distance between electrodes) for 45 min to 60 min.
- Following electrophoresis, stain the gel with appropriate amount of nucleotide / intercalating dye (e.g. ethidium bromide, GelRed Nucleic Acid or SYBR green) and visualize by using the corresponding technique. For more details, please refer to the Direction For Use of the dye.

5. Data Analysis – Validity and Qualitative Result

- The readily stained agarose gel must give discrete bands of expected sizes for control reactions and the reference DNA ladder. The actual PCR test analysis starts with the validity check of the entire PCR run. Therefore, check the results of Positive Control and Negative Control for presence / absence of expected product size of 210 bp. Afterwards, the DEV-specific status of each sample is analyzed by looking for presence / absence of the expected DEV-product size of 210 bp.

Test Evaluation

- The **PCR test run** is only **valid** if the Negative Control is negative and the Positive Control is positive with regard to the expected DEV-specific product size of 210 bp.
- A **sample** is DEV **positive**, if presence of expected DEV-specific product with size of 210 bp is observed.
- A **sample** is DEV **negative**, if absence of expected DEV-specific product with size of 210 bp is observed.

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