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For laboratory/veterinary use only, for *in vitro* diagnostic

CEV qPCR Kit

CEV qPCR Kit is intended to detect carp edema virus DNA in koi carp and common carp samples. The purified viral DNA is amplified using real-time PCR and detected using a FAM-labeled probe. The virus is identified based on a highly conserved p4a core protein gene fragment of the CEV genome. Additionally, the kit contains an endogenous internal control consisting of primers and a probe labeled with HEX dye for the carp glucokinase fragment. The internal control allows both extraction and amplification to be monitored.

Kit contents

Component	**Cat. no. E4363-01** 50 reactions of 25 µl	**Cat. no. E4363-02** 100 reactions of 25 µl
CEV Master Mix* brown tube	2 x 500 µl	4 x 500 µl
Positive Control** black cap	100 µl	200 µl
Water, nuclease free natural cap	500 µl	2 x 500 µl

* The CEV Master Mix includes: reaction buffer, dNTPs, primers and FAM-labeled probe for CEV, primers and HEX-labeled probe for the internal control, ROX reference dye and onTaq DNA Polymerase.

** Positive Control contains a synthetic DNA fragment of CEV.

Storage

The kit should be stored in the dark at −20°C. Avoid repeated thawing and freezeing (>2x), due to possible reduced sensitivity.

Types of DNA samples, DNA sample preparation

DNA from fish tissue samples, organs, ovarian/seminal fluids and cell cultures should be isolated using kits dedicated to the purification of viral DNA. Please follow the instructions recommended by the kit manufacturer. Due to the high sensitivity of the kit, both single and pooled samples of up to 10 individual samples can be tested.

The reference dye ROX

The passive ROX reference dye included in the master mix enables fluorescence normalization in particular cyclers. The use of ROX dye is required for all Applied Biosystems real-time PCR cyclers and optional for Agilent cyclers. ROX compensates for differences in fluorescence signal between wells caused by small differences in reaction volume and fluorescence fluctuations. ROX is not involved in the PCR reaction and does not interfere with real-time PCR on any instrument.

Procedure
Preparation of PCR reaction

Component	Negative amplification control -NAC	Negative extraction control-NEC	DNA sample	Positive Control CEV-TPC
CEV Master Mix	20 µl	20 µl	20 µl	20 µl
Water, nuclease free	5 µl	-	-	-
Purified water sample or negative sample CEV$^-$	-	5 µl	-	-
DNA Sample	-	-	5 µl	-
Positive Control	-	-	-	5 µl
Total volume	25 µl	25 µl	25 µl	25 µl

Notes:

1. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Thaw, gently vortex and briefly centrifuge all solutions. Keep the kit components on ice. Avoid multiple thawing and freezing (> 2x) of CEV Master Mix, as this may reduce the sensitivity. Set up PCR reactions at room temperature. The use of CEV Master Mix allows room temperature reaction setup.
3. Dispense 20 µl of CEV Master Mix into PCR tubes or plates.
4. Add:

- 5 µl of water (negative amplification control-NAC),
- 5 µl of water or negative sample CEV$^-$ purified according to DNA extraction protocol (negative extraction control-NEC),
- 5 µl of purified DNA (DNA sample),
- 5 µl of Positive Control CEV-TPC.

5. Centrifuge briefly to settle down the reaction components and remove bubbles. Place the samples in the cycler and start the program prepared according to the table below. Perform data analysis.

Real-time PCR Protocol

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	15 min	1
Denaturation	95°C	15 s	40
Annealing/Extention	60°C	60 s	

Notes:

1. Run PCR reactions using standard ramp rate (standard mode) only.
2. Fluorescence analysis in the FAM and HEX channels should be performed by the device at the end of the extension step. CEV DNA is detected in the FAM channel and carp glucokinase DNA is detected in the HEX channel.

Data Analysis and Interpretation

Sample type	FAM channel	HEX channel	Sample result
Negative amplification control-NAC	-	-	correct
Negative extraction control-NEC	-	-/+	correct
Positive Control CEV-TPC	+	+	correct
Sample 1	-	+	negative CEV^-
Sample 2	+	+	positive CEV^+
Sample 3	+	-	positive CEV^+
Sample 4	-	-	incorrect

Notes:

1. For the assay to be valid:
   - negative amplification control-NAC does not yield a signal in both FAM and HEX channels,
   - negative extraction control-NEC does not yield a signal in both FAM and HEX channels (for purified water) or yields a signal only in HEX channel (for negative sample CEV^-),
   - Positive Control CEV-TPC yields a signal in both FAM and HEX channels.
2. DNA sample is positive (CEV^+) and the assay is valid if:
   - the cryteria in the first point are met,
   - the sample yields a signal at least in FAM channel.

   Exceptionally very high concentrations of CEV DNA in the sample may lead to a reduced HEX signal or no HEX signal at all due to the competition with the endogeneous internal control.
3. DNA sample is negative (CEV^-) and the assay is valid if:
   - the cryteria in the first point are met,
   - sample yields a signal only in HEX channel.
4. A positive HEX signal for the sample means that the DNA extraction and amplification were successful. High C_T values (>35) for internal control may indicate partial PCR reaction inhibition. In turn, lack of a signal at all in the HEX and FAM channel simultaneously may indicate complete PCR reaction inhibition or that no DNA was added to the test sample. In this case it is recommended to dilute 10 times the DNA sample or repeat the DNA extraction and run the PCR reaction again.
5. Absence of a signal for the Positive Control CEV-TPC indicates to an error, which could be due to incorrect setup of the PCR reaction.