IQ Plus™ CEV Reagent Set

For Carp Edema Virus Detection

User Manual

***in vitro* use only**

2019/05

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INTENDED USE

IQ Plus™ CEV Reagent Set uses insulated isothermal polymerase chain reaction (iiPCR) technology (Chang *et al*., 2012; Tsai *et al*., 2012) to detect the specific nucleic acid sequence of tilapia lake virus (CEV). This reagent set is specially designed to be used on an iiPCR-compatible instrument, **POCKIT™** Nucleic Acid Analyzer. The intended users of the reagent set are aquaculture technicians who have basic laboratory skills.

This reagent set is intended for research purpose and *in vitro* use only.

SUMMARY AND EXPLANATION

Carp edema virus (CEV), also known as koi sleepy disease, is the causative agent of carp edema virus disease (CEVD). CEVD is an emerging disease of global concern that may cause high rates of morbidity and mortality in common carp and ornamental koi (Cyprinus carpio) (Stevens *et al*., 2018). The disease is characterized by typical sleepy behaviour, enophthalmia, generalized oedematous condition and gill necrosis (Lewisch *et al*., 2014).

PRINCIPLE OF THE PROCEDURE

The assay is based on iiPCR for qualitative detection of CEV. Fluorogenic probe hydrolysis chemistry is used to generate fluorescent signal when a specific DNA sequence of CEV is amplified. The primers and probe target specific sequences of CEV, and do not react with nucleic acids of tilapia and other pathogens.

PRODUCT DESCRIPTION

A. Materials Provided

1) **IQ Plus™** CEV Reagent Set (48 tests/package)

Component	Contents	Amount
CEV Premix Pack	■ Vials with lyophilized pellet containing dNTPs, CEV specific primers, fluorescent probes, and enzyme. ■ Desiccating agent pack.	6 individually sealed zip-lock packs (8 vials/pack)
Premix Buffer B	■ Reaction buffer to re-dissolve the lyophilized pellet.	2 vials (1.3 ml/vial)
CEV P(+) Control	■ Dried plasmid pellet containing CEV partial sequence as positive control.	1 vial
P(+) Control Buffer	■ Reaction buffer to re-dissolve the CEV P(+) Control.	1 vial (110 μl/vial)
Inoculating Loops		3 packs (20 pieces/pack)
User Manual		1 copy

2) **PetNAD™** Nucleic Acid Co-prep Kit

3) R-tube (48 tubes/pack)

B. Materials and Equipment Required, but Not Provided

1) **taco™** mini Automatic Nucleic Acid Extraction System (optional)
2) **POCKIT™** Nucleic Acid Analyzer: the iiPCR-compatible instrument for **IQ Plus™** Reagent Set
3) **cubee™** Mini-Centrifuge
4) Micropipette and filter tips
5) Phosphate buffered saline (PBS)

C. Storage and Stability

1) The reagent set should be stored at 4°C and is stable until the expiration date stated on the label.
2) Store Premix vials in sealed Premix Pack to avoid deliquescence of lyophilized components.
3) Reconstituted P(+) Control is stable for 6 months at 4°C. Aliquot reconstituted P(+) Control to avoid degradation of nucleic acid.

PRECAUTIONS

1) Do not open R-tube(s) after reaction to prevent any carryover contamination.

2) Perform extraction and amplification in two independent spaces to minimize contamination.

3) Do not reuse R-tube and Premix.

4) Include the P(+) Control to:

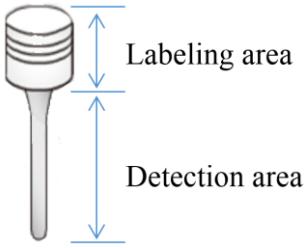
   - Ensure **POCKIT™** Nucleic Acid Analyzer is working.

   - Ensure reagent set performance after storage.

5) To get optimal fluorescence detection.

   - Wear powder-free gloves to handle R-tubes.

   - Do not label in the detection area of R-tube.



LIMITATION

1) **PetNAD™** Nucleic Acid Co-prep Kit or **taco™ mini** Automatic Nucleic Acid Extraction System is recommended for nucleic acid extraction.

2) The test should only be used for testing nucleic acid extracts. Do not add specimens directly into the Premix.

3) Any deviation from the recommended procedures may lead to sub-optimal results. Performance of the modified protocol should be validated by the users.

4) It is strongly recommended to use freshly prepared nucleic acids (within 1 hour after extraction) to achieve optimal results with **IQ Plus™** CEV Reagent Set.

SAMPLE PREPARATION

A. Sample Type

This reagent set is intended for analyzing nucleic acid extracted from the following recommended sample types:

Host	Carp only
Tissue type	Gill

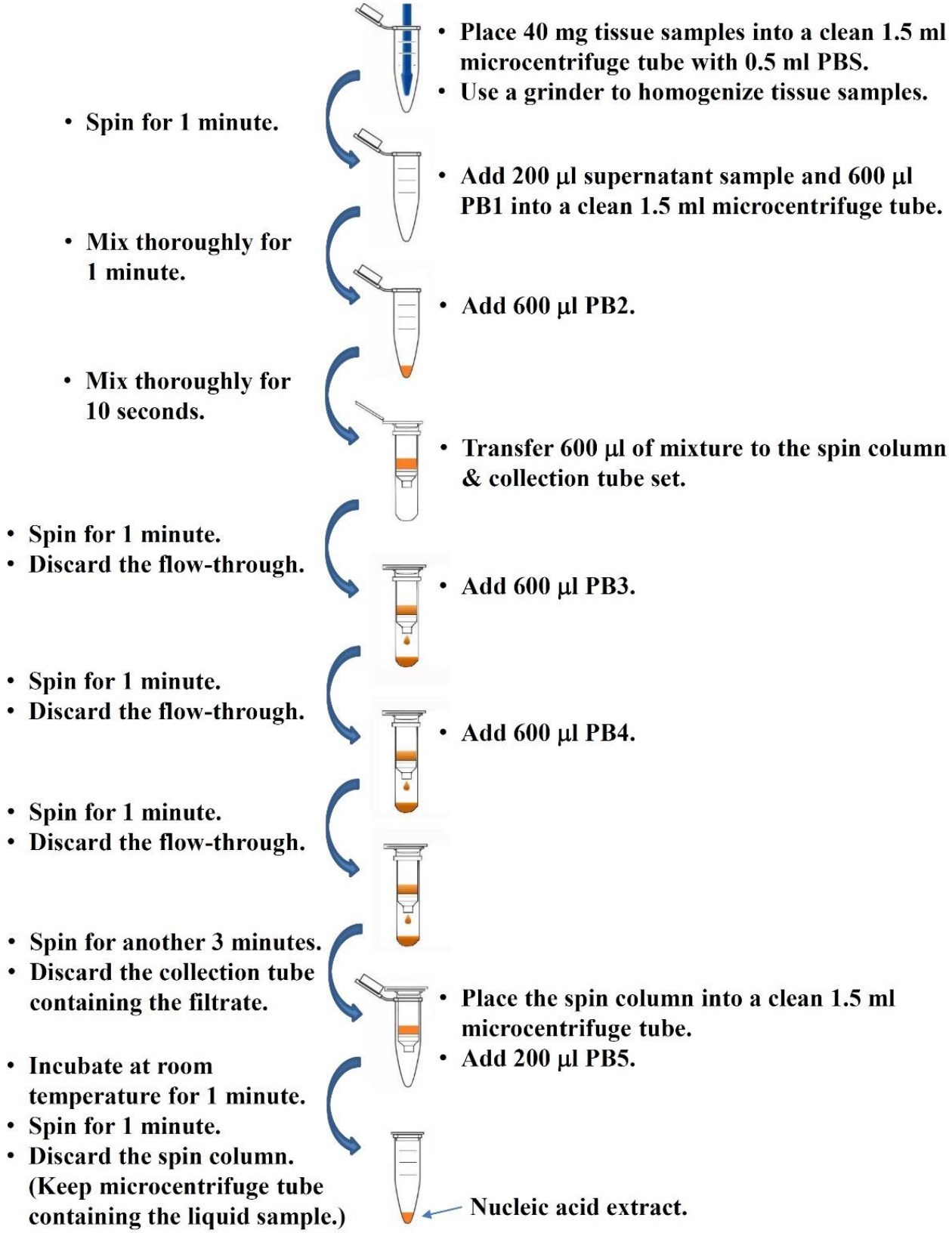
B. Using PetNAD™ Nucleic Acid Co-prep Kit

- **Note: Make sure to add 95% (or higher) ethanol to "PB2", "PB3" and "PB4" before first use.**

1) Sample pretreatment: Place 40 mg tissue samples into a clean 1.5 ml microcentrifuge tube with 0.5 ml PBS. Use a grinder (provided in **PetNAD™** Nucleic Acid Co-prep Kit) to homogenize tissue samples. Spin the tube for 1 minute in a **cubee™** Mini-Centrifuge.
2) Transfer 200 µl supernatant to a clean 1.5 ml microcentrifuge tube with 600 µl PB1.
3) Mix thoroughly for 1 minute.
4) Add 600 µl PB2 (with ethanol) into the tube.
5) Mix thoroughly for 10 seconds.

6) Place a spin column onto a collection tube.
7) Transfer 600 µl of the mixture to the spin column & collection tube set.
8) Spin for 1 minute and discard the flow-through from collection tube. Reassemble the collection tube to the spin column.
9) Add 600 µl PB3 (with ethanol) into the spin column & collection tube set.
10) Spin for 1 minute and discard the flow-through.
11) Add 600 µl PB4 (with ethanol) into the spin column & collection tube set.
12) Spin for 1 minute and discard the flow-through.
13) Spin for another 3 minutes to remove residual ethanol.
14) Transfer the spin column to a clean 1.5 ml microcentrifuge tube.
15) Add 200 µl PB5 into the spin column.
16) Incubate at room temperature for 1 minute.
17) Spin for 1 minute to elute the nucleic acids into the 1.5 ml microcentrifuge tube.
18) Discard the spin column and proceed to iiPCR analysis of the nucleic acid extract as soon as possible.

C. PetNAD™ Nucleic Acid Co-prep Kit Quick Guide



OPERATION PROCEDURE

- **NOTE: Before preparing the reactions for iiPCR testing, turn on POCKIT™ Nucleic Acid Analyzer to initiate the calibration for the instrument. The device will complete self-test within 5 minutes. Please refer to the user manual of POCKIT™ Nucleic Acid Analyzer for further details.**

- **NOTE: Before using for the first time, add 100 µl P(+) Control Buffer to P(+) Control. Store reconstituted P(+) Control at 4°C.**

1) Label R-tube(s) in the labeling area.

2) Prepare one Premix for each sample. (Premix tubes are in Premix Pack. Each Premix Pack contains eight Premix tubes.)

   - **NOTE: When the pellet is not found at the bottom of the tube, spin tube briefly to bring it down.**

3) Add 50 µl Premix Buffer B to each Premix tube.

4) Use the inoculating loop, take nucleic acid extracts or dissolved P(+) Control into each Premix tube. Spin Premix tubes briefly in a mini centrifuge (such as **cubee™** Mini-Centrifuge).

   - **NOTE: Please repeatedly dip the inoculating loop into solution three times to collect the correct solution volume.**

5) Transfer 50 µl Premix/sample mixture into R-tube.

6) Seal top of each R-tube with a cap. Make sure R-tube is capped tightly.

7) Place R-tube into the holder of **POCKIT™** Nucleic Acid Analyzer.

8) Spin tube/holder set briefly in **cubee™** Mini-Centrifuge to make sure all solution is collected at the bottom of R-tube.

- **NOTE: Make sure there are no bubbles in the solution.**
- **NOTE: Start reaction within 1 hour to prevent nucleic acid degradation (to prevent nucleic acid degradation and non-specific reaction).**

9) **POCKIT™** Nucleic Acid Analyzer reaction:

a) Select "520 nm".

b) When "System READY" is displayed, place the holder with R-tube(s) into the reaction chamber.

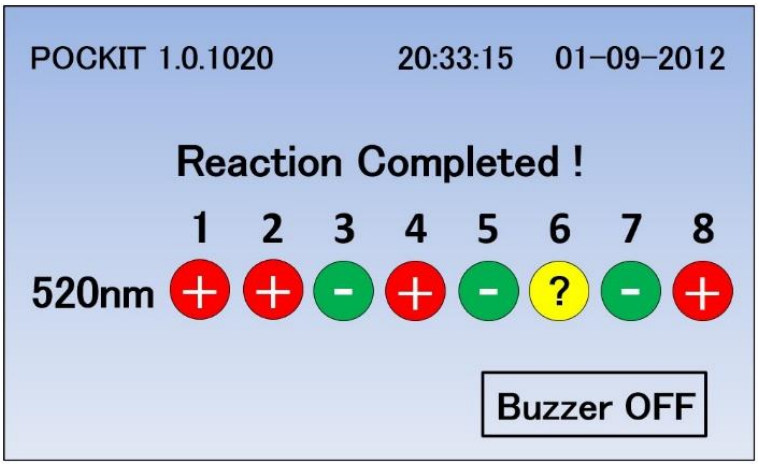
c) Tap cap of each R-tube to make sure the tube is positioned properly.

10) Close lid and press "Run" to start reaction program.

11) Test results are shown on the monitor after the reaction is complete.

DATA INTERPRETATION

*One example of results shown on the monitor.



520 nm	Interpretation
+	CEV positive
-	The nucleic acids of CEV are not detected.
?	Repeat reaction with freshly prepared nucleic acid.

ANALYTICAL SENSITIVITY

The detection limit of **IQ Plus™** CEV Reagent Set is up to 10 copies/ reaction.

TROUBLESHOOTING

Problems	Possible causes	Comments or solution
False Negative	1) Poor nucleic acid quality.	■ Check sample storage condition. ■ Please refer to Troubleshooting section of nucleic acid extraction kit user manual.
	2) Nucleic acid extraction failed.	■ Consult manual of nucleic acid extraction kit.
	3) No nucleic acid added	■ Please repeat the test.
	4) PCR inhibition.	■ Do not overload nucleic acid. ■ Spike 5 μl nucleic acid sample into a positive control reaction for a parallel PCR reaction. Negative results indicate the presence of inhibitors in the nucleic acid. In that case, prepare another nucleic acid extraction.
	5) Deterioration of the reagents.	■ Confirm the expiration date and storage condition. ■ The premix pellet should be kept dry, avoiding deliquescence.

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Problems	Possible causes	Comments or solution
False Positive	1) Contaminated micropipette.	■ Use aerosol free tips.
	2) Contaminated reagent.	■ Consult with **GeneReach** or local distributor.
	3) Contaminated laboratory or working area.	■ Consult with **GeneReach** about the guideline of lab contamination and cleanup.
	4) Reuse of micro-centrifuge tubes, tips, R-tubes and Premix.	■ Micro-centrifuge tubes, tips, R-tubes and Premix are for single-use only. Reusing these accessories would cause cross-contamination. ■ Used micro-centrifuge tubes, tips, R-tubes and Premix should be collected and discarded according to local regulation. Do not place the waste close to the working area to prevent cross-contamination.
Chemical leaks or spills into device.	1) Reaction tube (R-tube) broken or solution spilled into reaction chamber of **POCKIT™** Nucleic Acid Analyzer.	■ Consult with **GeneReach** or local distributor.

REFERENCE

1. Chang, H. G., Tsai, Y., Tsai, C., Lin, C., Lee, P., Teng, P., et al. (2012). A thermally baffled device for highly stabilized convective PCR. *Biotechnology Journal*, 7(5), 662-666. doi: 10.1002/ biot.201100453.

2. Lewisch, E., Gorgoglione, B., Way, K. and El-Matbouli, M. (2014). Carp Edema Virus/Koi Sleepy Disease: An Emerging Disease in Central-East Europe. *Transboundary and Emerging Diseases*, 62(1), pp.6-12.

3. Stevens, B., Michel, A., Liepnieks, M., Kenelty, K., Gardhouse, S., Groff, J., Waltzek, T. and Soto, E. (2018). Outbreak and treatment of carp edema virus in koi (Cyprinus carpio) from northern California. *Journal of Zoo and Wildlife Medicine*, 49(3), pp.755-764.

4. Tsai, Y., Wang, H. T., Chang, H. G., Tsai, C., Lin, C., Teng, P., *et al*. (2012). Development of TaqMan Probe-Based Insulated Isothermal PCR (iiPCR) for Sensitive and Specific On-Site Pathogen Detection. *PLoS ONE*, 7(9), e45278. doi:10.1371/journal.pone.0045278.