IQ Plus™ KHV Kit

For Koi Herpesvirus Detection

User Manual

***in vitro* use only**

2019/03

Manufacturer : GeneReach Biotechnology Corporation
Address : No. 19, Keyuan 2nd Road, Central Taiwan Science Park, Taichung City 407, Taiwan
TEL : 886-4-24639869
FAX : 886-4-24638255
Web Site : www.genereach.com

INTENDED USE

IQ Plus™ KHV Kit uses insulated isothermal polymerase chain reaction (iiPCR) technology to detect the DNA of koi herpesvirus (KHV) (Chang et al., 2012; Tsai et al., 2012). This kit is specially designed to be used on an iiPCR-compatible instrument, **POCKIT™** Nucleic Acid Analyzer. The intended users of this kit are aquaculture technicians who have basic laboratory skills.

This kit is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Koi carp is the main freshwater ornamental fish which exhibits very high degree of value addition. In recent years, the industry has been threatened by a newly recognized virus, Koi Herpesvirus (KHV) as episodes of KHV outbreaks have occurred in areas including Asia, United States and Europe. The spread of KHV has caused massive mortality in cultured common carp as well as in the ornamental koi carp. It is reported that KHV is killing four out of every five infected fish (Pearson, 2004). In Indonesia, KHV infection was reported to have affected over 5000 fish farmers and caused a loss of US$0.5 million within three months (Agus et al., 2002). The disease had also been reported in common or koi carp in 23 of Japan's 47 prefectures by the end of 2003 (Pearson, 2004). Such rapid spread of KHV is presumably resulted from worldwide trade and the trans-boundary movement when the fish is under the stress of transportation, handling, and the change of temperature (Agus et al., 2002).

GeneReach has developed **IQ Plus™** KHV Kit based on iiPCR technology, which is highly sensitive and specific for KHV detection. **IQ Plus™** KHV Kit is specially designed for on-site viral detection in the farm. The assay has been simplified for easy and fast operation with the use of compact and portable equipment for KHV detection at pond-side.

PRINCIPLE OF THE PROCEDURE

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

PRODUCT DESCRIPTION

A. Materials Provided

1) IQ Plus™ KHV Kit (48 tests/kit)

Component	Contents	Amount
KHV Premix Pack	■ Vials with lyophilized pellet containing dNTPs, KHV 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)
KHV P(+) Control	■ Dried plasmid pellet containing KHV partial sequence as positive control.	1 vial
P(+) Control Buffer	■ Reaction buffer to re-dissolve the KHV P(+) Control.	1 vial (110 μl/vial)
Inoculating Loops		3 packs (20 pieces/pack)
User Manual		1 copy

2) **IQ Plus™** Extraction Kit (50 tests/kit)

3) R-tube (48 tubes/pack)

B. Materials and Equipment Required, but Not Provided

1) **POCKIT™** Nucleic Acid Analyzer: the iiPCR-compatible instrument for **IQ Plus™** Kit

2) **cubee™** Mini-Centrifuge

3) Micropipette and filter tips

4) Phosphate buffered saline (PBS)

C. Storage and Stability

1) The kit 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 hydration 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:

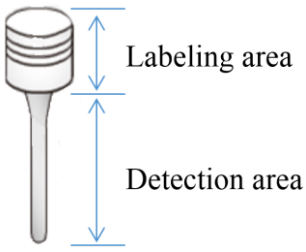
   - Ensure **POCKIT™** Nucleic Acid Analyzer is working.

   - Ensure kit 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) **IQ Plus™** Extraction Kit 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™** KHV Kit.

SAMPLE PREPARATION

A. Sample Type

This kit is intended for analyzing nucleic acids extracted from skin mucus of fish.

B. Using IQ Plus™ Extraction Kit

- **NOTE: Make sure to add 48 ml 95% (or higher) ethanol to "Solution 2" before first use.**

1) Moisten a sterilized cotton swab (cotton diameter ≤ ø 0.5 mm) with PBS. Use it to smear the surfaces of dorsal fin, collecting the skin mucus of fish.
2) Put the cotton swab into a 1.5 ml centrifuge tube with 500 µl Solution 1.
3) Mix well (or swirl the swab for 30 seconds in the centrifuge tube). Separate out the skin mucus sample in the solution.
4) Discard swab. Add 500 µl Solution 2 (with Ethanol) into the centrifuge tube.
5) Mix thoroughly and spin for 1 minute.
6) Transfer 500 µl supernatant to the spin column & collection tube set.
7) Spin for 1 minute and discard the flow-through from the collection tube. Reassemble the collection tube to the spin column.
8) Add 500 µl Solution 2 into the spin column & collection tube set.

9) Spin for 3 minutes and discard the flow-though.

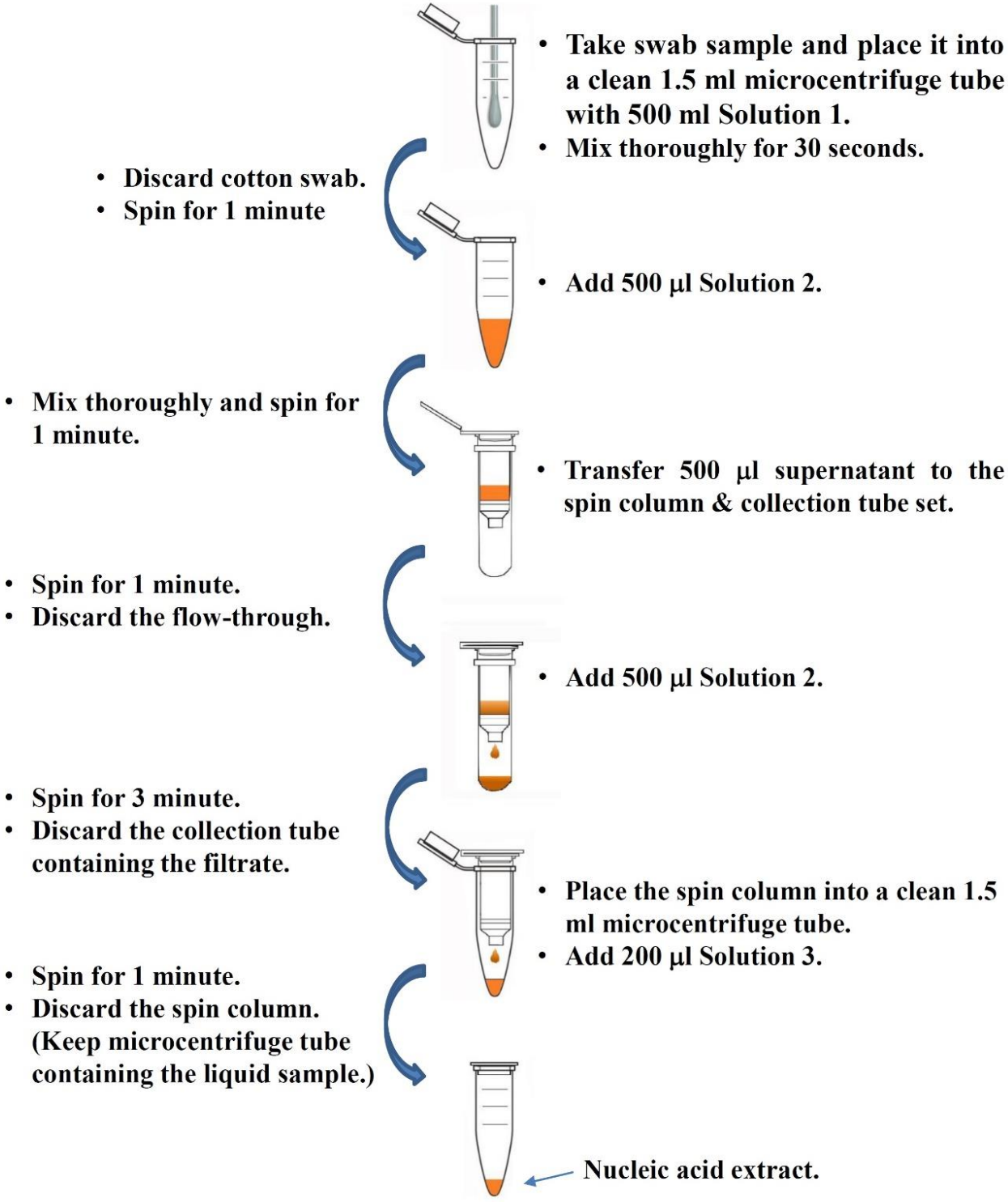
10) Transfer the spin column to a clean 1.5 ml microcentrifuge tube.

11) Add 200 µl Solution 3 into the spin column.

12) Spin for 1 minute to elute the nucleic acids into the 1.5 ml microcentrifuge tube.

13) Discard the spin column and proceed to iiPCR analysis of the nucleic acid extract as soon as possible.

C. IQ Plus™ Extraction 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.

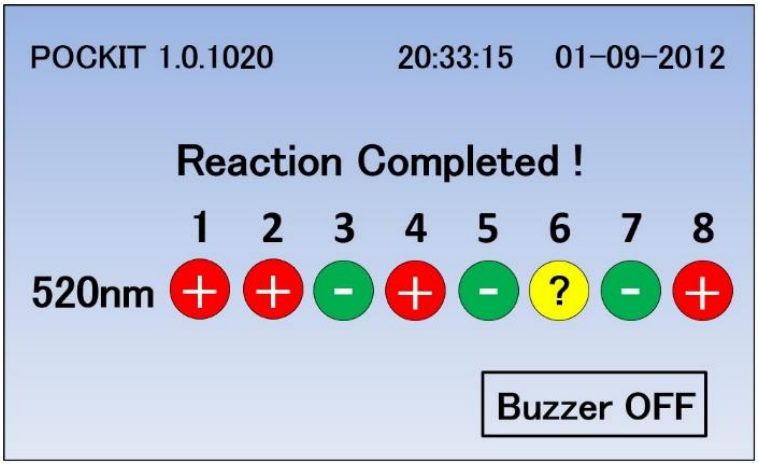
10) Close lid and press "Run" to start reaction program.

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

∗ Please refer to Operation Guide on GeneReach website.

DATA INTERPRETATION

*One example of results shown on the monitor.



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

ANALYTICAL SENSITIVITY

The detection limit of **IQ Plus™** KHV Kit 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 **IQ Plus™** 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.

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. Agus, S. et al. (2002) Field investigations on serious disease outbreak among koi and common carp (*Cyprinus carpio*) in Indonesia. 5th Symposium on Diseases in Asian Aquaculture.

2. 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.

3. Pearson, H. (2004) Carp virus crisis prompts moves to avert global spread. Nature 427: 577

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.