POCKIT Nucleic Acid Analyzer

USER MANUAL

Validated and certified by the OIE as fit for the purposes defined in this kit insert provided with this kit. Registration number 20130108

Manufacturer:
GeneReach Biotechnology Corporation
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2014/07

CONTENT

Symbols

	**Date of manufacturing**	
	**Manufacturer**	
	**Catalogue number**	
	**Serial number**	
	**Alternative current**	
	**Consult instruction before use**	
	**Caution**	Risk of personal injury to the operator or a safety hazard to the surrounding area.
	**WEEE symbol**	This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC.

Section 1. Introduction

The **POCKIT™** Nucleic Acid Analyzer (**POCKIT**) is designed for performing insulated isothermal polymerase chain reaction (iiPCR). It provides iiPCR-based analysis and is equipped with two optical channels (520 nm, 550 nm) for multiplex detection. The qualitative test results are readily displayed on the LCD monitor, and automatically saved in a SD card. It generates results for up to 8 nucleic acid samples within 1 hour.

1.1 Intended Use

POCKIT is intended to provide qualitative detection of nucleic acid targets using fluorescence-based iiPCR reagents. It is intended for *in vitro* use only and for research purposes.

Section 2. General Information

2.1 Product Components

- **POCKIT™** Nucleic Acid Analyzer × 1 unit
- Power Cord × 1 piece
- User Manual × 1 copy
- Warranty Card × 1 copy
- Holder × 1 piece

2.2 Specifications

Dimensions: 280 mm (W) × 250 mm (D) × 85 mm (H)
Net Weight: 2.1 kg
Power Supply: 100-120/200-240 V AC, 50/60 Hz, 2A
Fuses: 2A, 250V AC, ϕ 5 × 20 mm fast-acting, low breaking capacity glass tube fuses.
Operating Temperature: 15 ~ 35℃

2.3 Testing Capacity

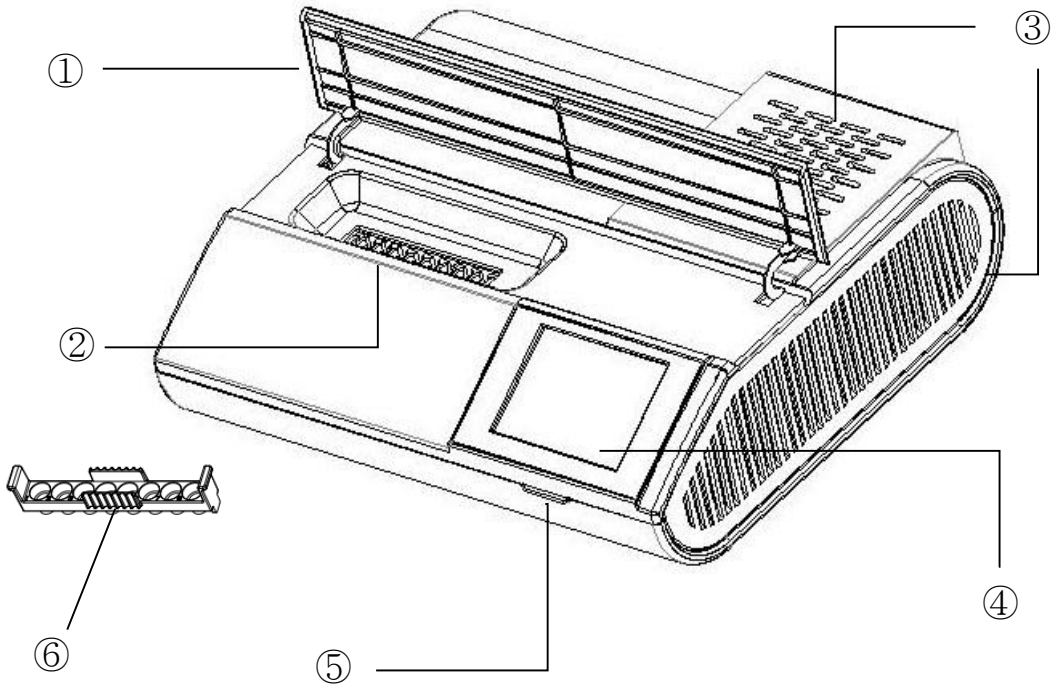
Number of Samples: 1 ~ 8 reactions per run
Reaction Volume: 45 ~ 55 μl per reaction

2.4 Equipments and Materials Required But Not Provided

- Nucleic acid extraction kit
- Nucleic acid amplification reagents and kits
- 200 μl and 1000 μl micropipette, tips, and 1.5 ml tubes
- R-tubes and Caps
- **cubee™** mini-centrifuge
- Disposable gloves

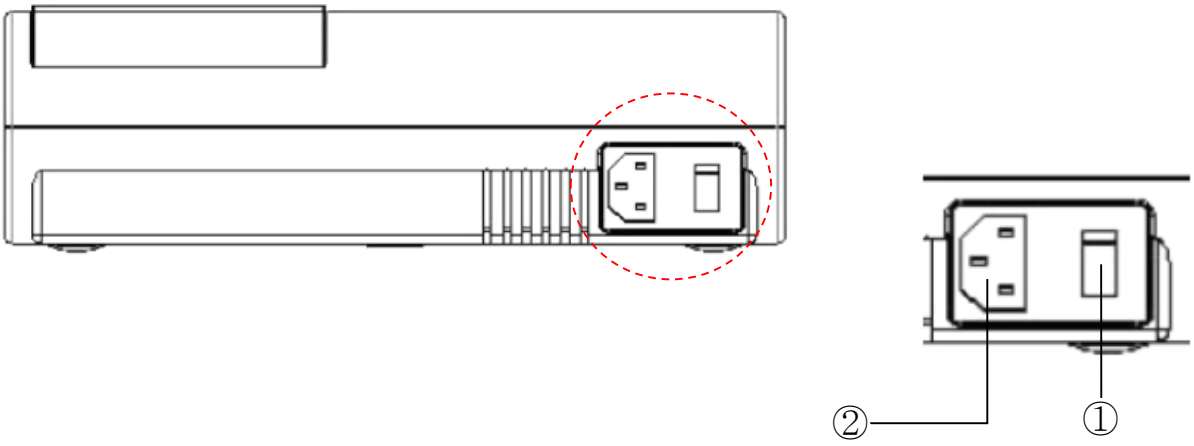
*__Note__: To avoid power failure, connecting **POCKIT** to an uninterrupted power system (UPS) and/or voltage regulator is highly recommended.

2.5 Front View



No	Item	Description
1	Lid	Isolates the heat source, and prevents light interference on the reaction chamber.
2	Reaction well	Accommodate the R-tube holder to perform the amplification.
3	Cooling vents	Releases the heat generated during reaction.
4	Control panel	A touchscreen where users key in the operation commands and view the results.
5	SD card slot	A slot where users insert the SD card to run **POCKIT** and save reaction raw data.
6	Holder	Load R-tubes into the reaction wells.

2.6 Rear View



No.	Item	Description
1	Main switch	To turn on/off the main power
2	Socket	To connect to the power cord

Section 3. Important Notes

Check the device upon receiving. If any items are missing or harmed, please contact your local distributor or GeneReach immediately for replacement. Do not use any damage items as they may lead to poor performance of the test or harm the user.

3.1 Safety Information

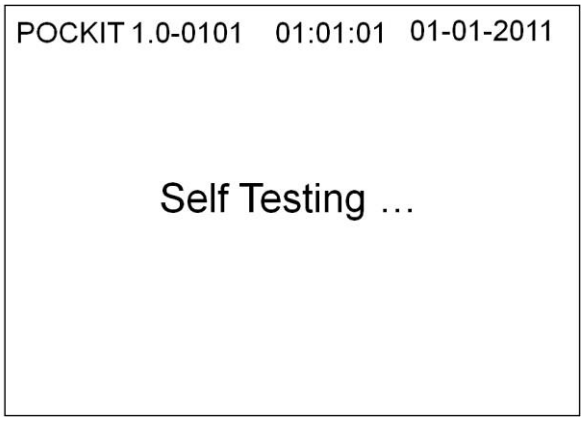
	DO NOT OPEN LID DURING REACTION

- Do not touch any plug and electric switch with wet hands.
- This product should be placed in a dry, clean and ventilated indoor place. Strictly keep away from moisture.
- Switch off and unplug the device before moving it.
- Hold the plug while pulling the power cord out from the electrical outlet. Do not pull it by the cord directly.
- Do not place the power cord on any hot surfaces.
- Pack the device carefully before moving and delivering. Vibration or crash may cause damage.
- Users should revalidate the device after it has been moved.
- Do not look directly into the LED light in the reaction wells.
- Only use consumables and accessories provided from GeneReach.
- Make sure the ambient temperature during reaction is kept at 15 ~ 35℃.
- SD card should not be removed until the device is switched off.
- Do not disassemble the device if any problem occurs. Contact your local distributor or GeneReach directly for troubleshooting.
- In case of emergency or abnormal conditions, turn off the power switch or unplug. Do not block access to power switch or plug.
- When working around the device, always wear a lab coat, disposable gloves and protective goggles.
- Waste materials, such as gloves and R-tube, may be biohazardous. Please discard them carefully according to your local regulation.

Section 4. Operation

4.1 Switch On

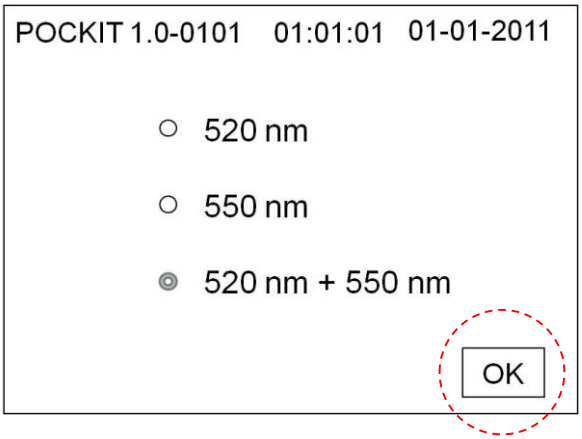
Plug in the **POCKIT** and switch on the main power located in the back. The device will complete self-testing within 5 minutes.



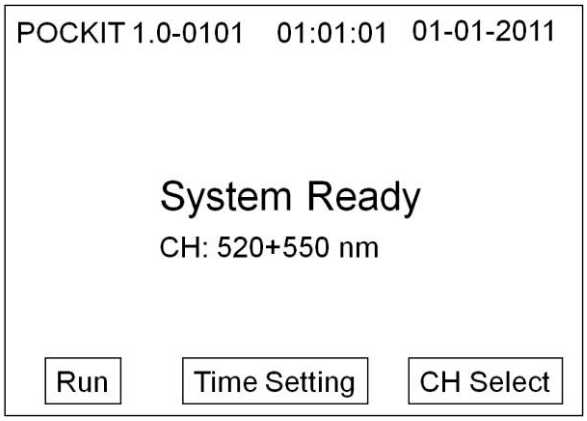
*__Note__: If self-testing runs longer than 5 minutes, please see Troubleshooting.

4.2 Choose Detection Wavelength

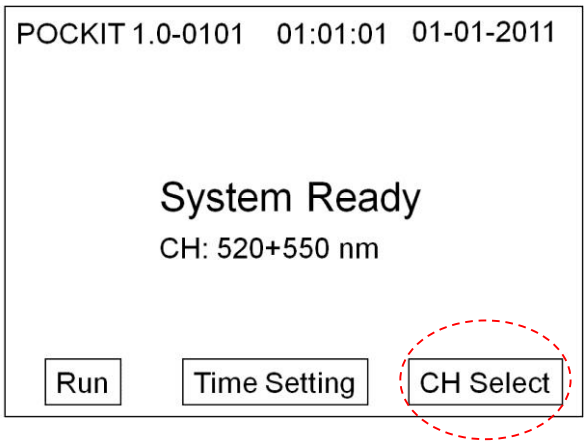
1) After self-testing, choose the detection wavelength according to the kits or methods used and press "**OK**".



2) “**System Ready**” will be shown after choosing the detection wavelength.

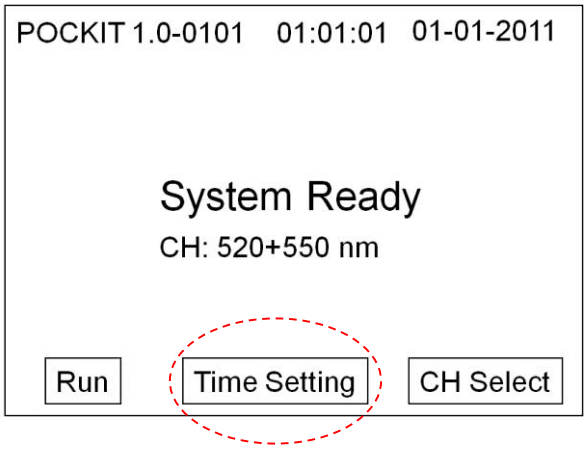


3) Before the reaction starts, to change the detection wavelength, press “**CH Select**” and choose again.



4.3 Set up Date and Time

1) Press "**Time Setting**".



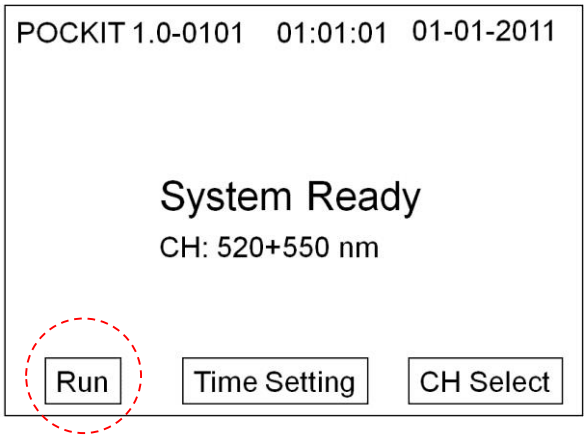
2) Touch the numbers that needs to be changed on the screen. Press "**Up**" and "**Down**" buttons to select the desired number. Press "**OK**" to confirm and return to the "**System Ready**" screen. Press "**Cancel**" to quit and leave the "**Time Setting**" screen.

*__Note__: The data derived from each test is stored in the SD card and in a folder named after the device's default date and time when the user switches on the analyzer.

4.4 Run iiPCR Reaction

1) Place R-tubes into a holder.

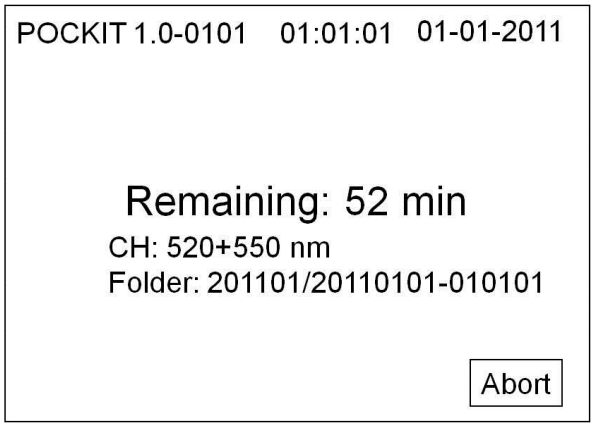
2) Place the holder with R-tubes into the reaction well. Close the lid and press "**Run**" to start the reaction.



*__Note__: Ensure the holder with R-tubes completely fits into the reaction wells.

⚠ **Caution**: Do not look directly into LED lights in the reaction wells.

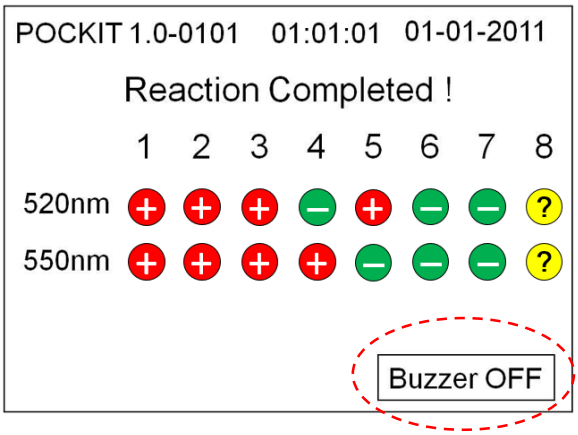
3) The screen will display the remaining reaction time and the folder name of the reaction.



⚠ **Caution**: Should any of the circumstances below occur, the results are unreliable:

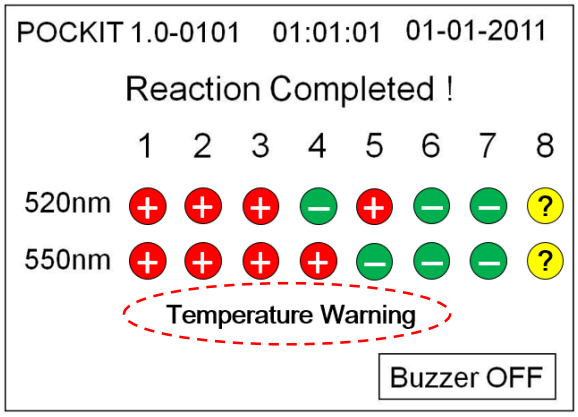
- The lid is opened or the SD card is removed during the **POCKIT** reaction.
- The ambient temperature is not in the range of 15-35℃ during the reaction.
- The variation of ambient temperature is greater than 5℃ during the reaction.

4) When the reaction complete, a beeping tone is made from **POCKIT**. Press "**Buzzer OFF**" to turn off the sound.



*__Note__: If there is no beep sound after the completion of the reaction, please see Troubleshooting.

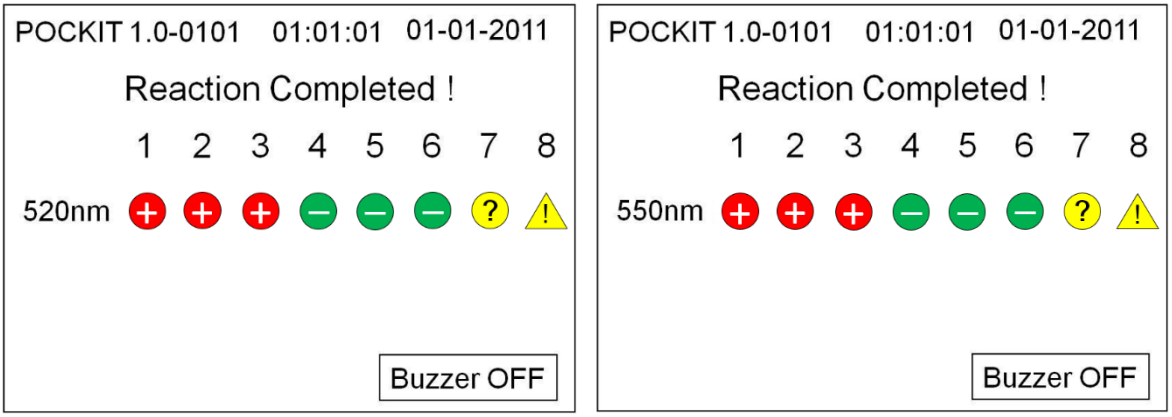
5) If the reaction temperature is abnormal during the reaction, it will show "**Temperature Warning**" on the screen.



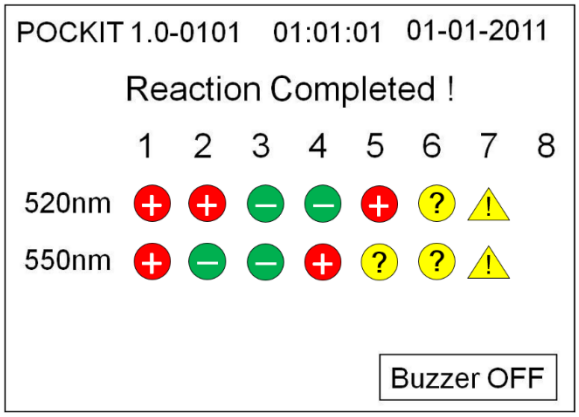
⚠ **Caution:** Fluctuation of ambient temperature should not be more than 5°C during the reaction.

4.5 Display the Result

1) An example of results displayed at the end of a single detection wavelength (either **520 nm** or **550 nm**) program.



2) An example of results displayed at the end of a double detection wavelength (**520 nm + 550 nm**) program.



3) Description of the results:

Results	Description
⊕	Positive
⊖	Negative
?	Undetermined
⚠	Warning

*__Note__: Please interpret the results according to the user manual of the kits used.

*__Note__: When you see a "⚠" symbol, please contact your local distributors or **GeneReach** for assistance.

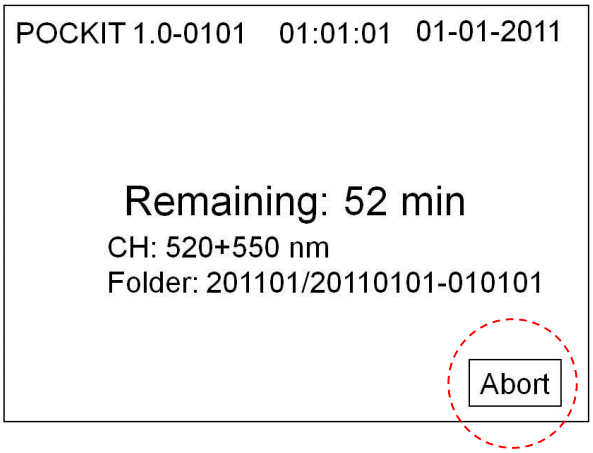
4.6 Review the Raw Data

1) Insert the SD card on a PC or Mac. Find the **raw.csv** file in the folder of the reaction.

2) Please refer to the kit manual for data interpretation.

4.7 Exit the Experiment

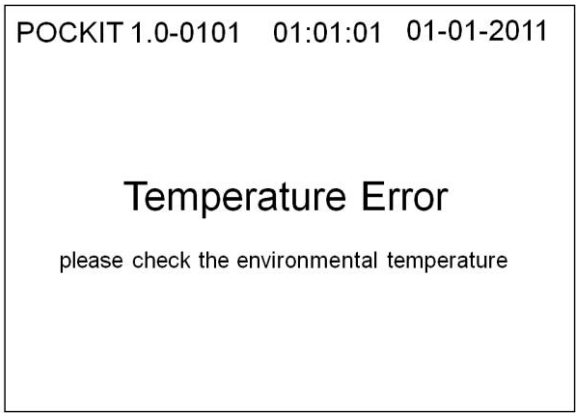
1) Press "**Abort**" to pause the reaction.

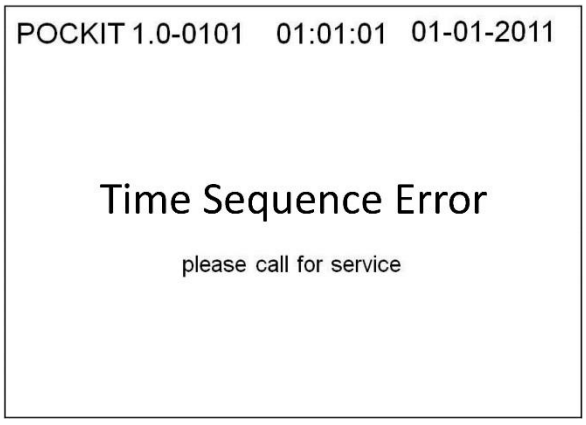


2) Press "**Yes**" to confirm and return to the self-testing screen, or press "**No**" to continue the reaction.

4.8 Errors occurred during the operation

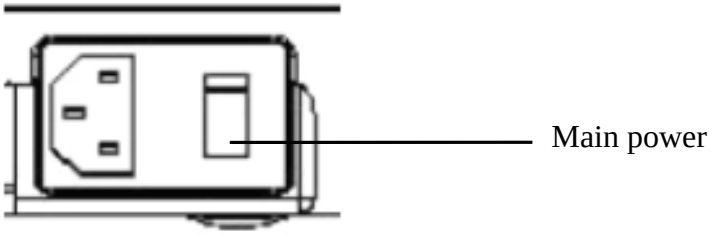
If any Errors listed below are shown, please see Troubleshooting.





4.9 Switch Off

After the reaction, switch off the main power and unplug the SD card to read the raw data on a PC or Mac.



⚠ **Caution**: Ensure the reaction has been completed before shutting down the device.

Section 5. Cleaning and Maintenance

5.1 General Care

Use only clean and wet paper towel or cotton to wipe out stains in the reaction chamber. Do not disassemble the **POCKIT** for cleaning as it will void the warranty. Do not use solvent, detergent or bleach to clean the **POCKIT** surface.

*__Note__: Always wear gloves, and always switch off and unplug the **POCKIT** before cleaning.

5.2 Dealing with Spills

1) Wipe out visible spills with disposable paper towel immediately, and contact your local distributor or GeneReach directly for further assistance.

2) Please use ultraviolet lights (UV) to irradiate and sterilize **POCKIT** for 15 minutes.

Troubleshooting

Observation or Problems	Comments and Suggestions
(a) Self test runs more than 5 minutes	■ Re-start the device. ■ Contact your local distributors or GeneReach for assistance.
(b) System crash	■ Re-start the device, prepare new reagents in R-tube, and repeat the reaction. ■ If any further problems occur, please contact your local distributor or GeneReach for assistance.
(c) SD card error	■ Re-insert SD card, or restart device. ■ Contact your local distributor or GeneReach for assistance if the problem persists.
(d) No display on screen	■ Make sure the plug has been connected to the power system. ■ Replace fuse. ■ Contact your local distributor or GeneReach for assistance.
(e) System error	■ Contact your local distributor or GeneReach for assistance.
(f) Temperature error	■ Keep ambient temperature between 15-35℃ during reaction. ■ Contact your local distributor or GeneReach for assistance.
(g) Camera error	■ Contact your local distributor or GeneReach for assistance.
(h) Time sequencing error	■ Contact your local distributor or GeneReach for assistance.
(i) Create data directory error	■ Check storage space is available in SD card. ■ Keep number of files/folders below 512 in each directory. ■ Contact your local distributor or GeneReach for assistance.
(j) No beep sound	■ Contact your local distributor or GeneReach for assistance.
(k) "⚠" symbol shown after the run	■ Contact your local distributor or GeneReach for assistance.

Appendix

OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: IQ Plus™ WSSV Kit with POCKIT System **Manufacturer**: Genereach Biotechnology Corporation **OIE Approval number**: 20130108 **Date of Registration**: May 2013

Disease: White Spot Disease
Pathogen Agent: White Spot Syndrome Virus (WSSV)
Type of Assay: Insulated isothermal Polymerase Chain Reaction
Purpose of Assay: Certified by the OIE in May 2013 as fit for the detection of white spot disease in target tissues (Shrimp tissue of ectodermal and mesodermal origin) of *Litopenaeus vannamei* and for the following purposes:

1) To certify freedom from infection (<10 virions/reaction) in individual animals or products for trade/movement purposes;
2) To confirm diagnosis of suspect or clinical cases (confirmation of a diagnosis by histopathology or clinical signs);
3) To estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control).

Species and Specimen: *Litopenaeus vannamei*; Shrimp tissue of ectodermal and mesodermal origin.

A. Information on the kit

Information can be found by emailing: sales@genereach.com,
or by visiting http://www.iq2000kit.com/products_2.php?bgid=3&gid=6&sgid=34.

In summary, IQ Plus™ WSSV Kit with POCKIT System was designed for qualitative detection of WSSV DNA based on multiplex insulated isothermal PCR technology (iiPCR; Chang *et. al.*, 2012; Tsai *et. al.*, 2012a; Tsai *et. al.*, 2012b). IQ Plus™ WSSV Kit with POCKIT System is designed to be used with a compact and portable iiPCRcompatible instrument, POCKIT™ Nucleic Acid Analyzer (POCKIT™). IQ Plus™ WSSV Kit with POCKIT System is highly sensitive and specific for WSSV DNA detection from aquaculture specimen and suitable for onsite viral DNA detection. Specific primers and probe (520-nm fluorescent signal) are designed to detect WSSV DNA in samples. In addition, internal control (IC) primers and probe (550-nm fluorescent signal) are used to target a house-keeping gene of *Penaeid* shrimps. The assay has been simplified for easy and fast operation in POCKIT™ for pond-site WSSV DNA detection.

B. Summary of validation studies

1) Analytical characteristics

■ ***Repeatability*:**

Various types including negative and positive WSSV-infected shrimps (*L. vannamei*) were selected and three production batches were tested. Each sample was tested in quadruplicates per run. The data showed 100% agreement among the test results.

■ ***Analytical specificity*:**

WSSV-, Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)-, or Hepatopancreatic Parvovirus (HPV)-infected *L. vannamei* were used to test the specificity of IQ Plus™ WSSV Kit with POCKIT System. The IHHNV- and HPV-infected samples were confirmed to be WSSV-negative by IQ 2000™ WSSV Detection and Prevention System (DPS). Signals were generated from only WSSV-infected, not from IHHNV- and HPV-infected samples in IQ Plus™ WSSV Kit with POCKIT System.

■ ***Analytical sensitivity*:**

Analysis using standard plasmid (pWSSV1) and purified WSSV genomic DNA of known copy numbers shows that the ≥ 95% detection rate of IQ Plus™ WSSV Kit with POCKIT System was 23.7 and 16.9 copies pWSSV1 and WSSV DNA, respectively, per reaction. Furthermore, analysis of DNA extracted from WSSV-infected *L. vannamei* serially diluted with ddH2O or DNA extracts of SPF *L. vannamei* shows that the detection endpoint (10^4 dilution) of IQ Plus™ WSSV Kit with POCKIT System was similar to that of IQ2000™ WSSV DPS.

2) Diagnostic Characteristics

■ ***Test Cut-off Determination:***

IQ Plus™ WSSV Kit with POCKIT System, based on iiPCR and fluorescent probe detection principles, is designed to work in an iiPCR-compatible instrument, POCKIT™. The cut-off for POCKIT™ device were determined on the basis of fluorescent signal of numerous NTC and positive reactions of iiPCR assays developed for various targets at GeneReach (confidential data).

Readouts of the results are determined as follows:

- When "+" is displayed on POCKIT™, the sample is classified as WSSV positive.
- When "-" is displayed on POCKIT™, the sample is classified as WSSV negative.
- When "?" is displayed on POCKIT™, the test result is indeterminate and should be repeated.

■ ***Diagnostic sensitivity (DSn) and specificity (DSp) estimates***

Diagnostic sensitivity was evaluated by comparing the test results of IQ Plus™ WSSV reaction of positive reference animals which were identified by IQ2000™ WSSV DPS. Pleopods of 400 WSSV-positive samples were sampled and tested in this study. Negative reference animals

selected by IQ2000™ WSSV DPS were also subjected to analysis by IQ Plus™ WSSV Kit with POCKIT System.

		IQ2000™ WSSV DPS	
		Positive	Negative
IQ Plus™ WSSV Kit with POCKIT System	Positive	374	9
	Negative	26	291

In summary, this validation testing was conducted on 700 samples. The results are: sensitivity: 93.5% [95% confidence interval (CI): 90.61–95.56%], specificity: 97.0% [95% CI: 94.31–98.50%].

■ ***Comparative performance***

See "*Diagnostic sensitivity (DSn) and specificity (DSp) estimates".*

■ ***Agreement and discrepancies***

The results revealed that when compared to IQ2000™ WSSV DPS, with defined reference animals, the diagnostic sensitivity of IQ Plus™ WSSV Kit with POCKIT System was 93.5% with a 95% CI of 90.61% - 95.56%, and the diagnostic specificity was 97.0% with a 95% CI of 94.31% - 98.50%. In addition, with un-defined reference animal, IQ Plus™ WSSV Kit with POCKIT System showed 100% agreement (100/100) for both sensitivity and specificity. For this experiment, pleopods of 100 un-defined shrimps obtained randomly from a local farm were sampled and examined by both IQ2000™ WSSV DPS and IQ Plus™ WSSV Kit with POCKIT System. Statistical analysis using one-tailed binomial test suggested that results from these two experiments (defined reference animals and un-defined animals) agreed with each other.

3) Reproducibility

Different lots of IQ Plus™ WSSV Kit with POCKIT System were sent to three different labs located in Chinese Taipei and USA (including two OIE Reference Laboratories) to be tested. Trunk muscles of total of 64 *L. vannamei* samples were aliquoted, preserved in 95% ethanol, and sent to all three laboratories. Each sample was analysed with 3 batches of IQ Plus™ WSSV Kit with POCKIT System. Chi-square test for homogeneity was conducted to analyse the experimental results generated from the three labs.

The results showed that there was no difference among different laboratories with three batches of IQ Plus™ WSSV Kit with POCKIT System.

4) Applications

The kit is being used worldwide by different laboratories (Private and Public)

C. References

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3. Chang, P.S. et al. (1996). Identification of white spot syndrome associated baculovirus (WSSV) target organs in shrimp, *Penaeus monodon* by in situ hybridization. Dis Aquat Org 131-139.

4. Chou, H.Y. et al. (1995). Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. Dis. Aquat. Org. 23: 165-173.

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6. Lightner, D.V. (ed.) (1996). A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Soc., Baton Rouge.

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8. Lo, C.F. et al. (1996a). Detection of baculovirus associated with white spot syndrome (WSSV) in penaeid shrimps using polymerase chain reaction. Dis. Aquat. Org. 25: 133-141.

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18. Alan Agresti, Categorical Data Analysis (2nd edition), Wiley Press, 2002.