POCKIT™ *Toxoplasma gondii* Reagent Set

(Catalog # apls-083)

I. Introduction

POCKIT™ *Toxoplasma gondii* Reagent Set provides an effective tool for sensitive detection of the nucleic acids of *Toxoplasma gondii*. POCKIT™ *Toxoplasma gondii* Reagent Set uses insulated isothermal polymerase chain reaction (iiPCR) technology to detect the nucleic acids of *Toxoplasma gondii* (Chang *et al.*, 2012; Tsai *et al.*, 2012). Fluorogenic probe hydrolysis chemistry is used to generate fluorescent signal when a specific amplicon is amplified. The primers and probe target specific sequences of *Toxoplasma gondii*, and do not react with host genomic DNA or nucleic acids of other pathogens.

II. Intended use

This reagent set is specifically designed to be used on iiPCR-compatible instruments, POCKIT™ Nucleic Acid Analyzer. This reagent set is intended for research use purpose.

III. Product description

A. Materials Provided

1) POCKIT™ Reagent Set, (48 tests/package)

Component	Contents or Purpose	Amount
Premix Pack	■ *Toxoplasma gondii* Premix (lyophilized pellet) containing dNTPs, primers, probe, and enzyme for amplification. ■ Desiccating agent pack.	6 bags (8 *Toxoplasma gondii* Premix vials and 1 desiccant/bag)
Premix Buffer B	■ Reaction buffer to re-dissolve the lyophilized pellet.	2 vials (1.3 ml/vial)
P(+) Control	■ Dried plasmid containing partial sequence of *Toxoplasma gondii* as positive control.	1 vial
P(+) Control Buffer	■ Reaction buffer to re-dissolve P(+) Control.	1 vial (110 µl/vial)
User Instruction		1 copy

2) R-tube , 48 tubes/pack

B. Materials and Equipment Required, but Not Provided

1) PetNAD™ Nucleic Acid Co-prep Kit or taco™ mini Automatic Nucleic Acid Extraction System (optional)
2) POCKIT™ Nucleic Acid Analyzer: the iiPCR-compatible instrument.
3) cubee™ Mini-Centrifuge (cubee)
4) Micropipette and tips

IV. Suggested sample type for extraction

Feline feces.

V. Limitations

A. The test should only be used for testing purified nucleic acid (extracts). Do not add specimens directly into the Premix.
B. PetNAD™ Nucleic Acid Co-prep Kit or taco™ mini Automatic Nucleic Acid Extraction System is recommended for nucleic acid extraction.
C. Any deviation from the recommended procedures may lead to sub-optimal results. Performance of the modified protocol should be validated by users.
D. It is strongly recommended to use freshly prepared nucleic acids (within 1 hour after extraction) to achieve optimal results.
E. The reagent set should be stored at 4°C and is stable until the expiration date stated on the label.

VI. Operation procedure

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

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

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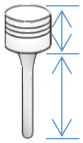
1) Label R-tube(s) in the label area.
2) Prepare one Premix for each sample. (Premix tubes are in Premix Pack. Each Premix Pack contains eight Premix tubes.)



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 into each Premix tube.
4) Add 5 µl nucleic acid extract or P(+) Control to each Premix tube. Mix by pipetting up and down. Spin Premix tubes briefly in a mini centrifuge (such as cubee).
5) Transfer 50 µl Premix/sample mixture into the 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.
8) Spin tube/holder set briefly in cubee to make sure all solution is collected at the bottom of R-tube.

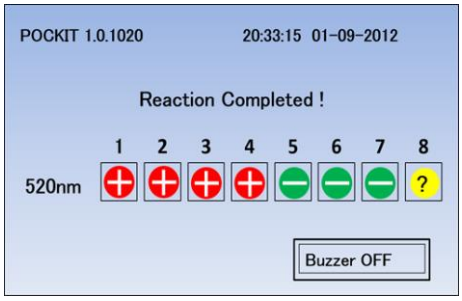
Note: Make sure there is no bubble in the solution.

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 "520nm".
   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.

VII. Data interpretation

One example of results shown on the monitor.



520 nm	Interpretation
⊕	Positive
⊖	Negative
?	Repeat reaction with freshly prepared nucleic acid.

VIII. Analytical sensitivity

The detection limit is about 10 copies/reaction.

IX. Reference

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

Tsai, Y., *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

Declaration

This is to declare this product that is designed and produced by GeneReach is not animal product or reagent toxic to humans. In addition, the package does not contain any live reagents, is inodorous, non-poisonous, non-hazardous, non-infectious, non-corrosive, non-inflammable, non-offensive to human health. GeneReach shall assume all responsibility for the declared statements detailed above.

For any questions, please contact GeneReach Technical Service Department: Technical-Service@genereach.com