IPA™-Green Instructions for Use

1. Application

The IPA™-Green reagent system is an isothermal amplification technology platform that is innovatively designed and developed for amplification and detection of DNA templates when combined with our RapidDetector™ technology.

The RapidDetector™ is a highly sensitive fluorescence mini-device (320 grams) making it highly portable and easy to use!. The device output is a plus sign (+) as positive for the target, or a minus sign (-) as negative for the target after 20 minutes at a continuous temperature of 42 °C.

2. IPA™-Green Kit (50 tests)

The IPA™-Green Kit is a reagent system for amplification and qualitative detection on the RapidDetector™. Successful reaction is achievable in a single tube after addition of all the IPA-Green™ reagents. The kit contains all the required components for a successful detection of amplified DNA, shown in T1:

T1: IPA^{TM}-Green Kit components

Component	Number of vials	Volume (µL/vial)	Number of total tests
IPA^{TM}-Green Master Mix	1	1120	50
IPA^{TM}-Green Primers Mix	1	80	50
*IPA^{TM}-Green Positive Control	1	50	50
*IPA^{TM}-Green Negative Control	1	50	50

***Avoid repeated freezing and thawing. The controls are non-infectious and should be used along with the primers mix. Be careful not to contaminate your reagents and materials with the controls and avoid cross-contamination between tubes. Repeated freezing and thawing and improper storage can degenerate the positive control.**

3. Storage of the IPA^{TM} Green kit

The IPA^{TM}-Green Kit should be stored at -20°C on arrival. If tubes are compromised on arrival, contact customerservice@isobiotech.com.au immediately. All components should be stored according to the instructions on the labels. Where possible, avoid repeated thawing and freezing of all the kit reagents including the controls as efficiency of the kits might be compromised. The reagents should be frozen in aliquots if they are to be used intermittently. Once thawed, kit components should be used within two hours so that kit components do not degrade and efficacy of the kit remains uncompromised.

4. Protocol

Prepare PCR primer stock in nuclease-free water and store at –20°C for up to 1 - 2 years.

Thaw all components to be used at room temperature and place on ice. Vortex briefly to mix and centrifuge to collect material. Prepare IPA™–Green reaction mix as described in T2:

T2: IPA™–Green reaction mix preparation

Component's	DNA target detection	No Template Control (NTC)
IPA™ Green Master Mix	22.4 µL	22.4 µL
PCR Forward primer (10 µM)	0.8 µL (0.32 µM)	0.8 µL (0.32 µM)
PCR Reverse primer (10 µM)	0.8 µL (0.32 µM)	0.8 µL (0.32 µM)
DNA template (1 -1 00 ng)	1 µL	-
Sterile Water	-	1 µL
Final volume	25 µL	25 µL

Alternate reaction setup: For ease and less pipetting, we recommend making working stock of the PCR primers by mixing equal volume of both forward and reverse primers at a usable concentration of 10 µM, mix and add 1.6 µL (0.64 µM).

- Vortex reaction mix and centrifuge to collect material.
- Pipet 24 µL per reaction into PCR tube and add sample. Mix by vortexing and centrifuge to collect.
- Close the PCR tube properly.
- Incubate in the RapidDetector™ at 42 °C for 20 minutes.

If reaction products will be manipulated or analysed after reaction is complete, inactivate by heating at > 80°C for 10 minutes.

Caution: Like any other nucleic acid amplification technology, you need to prevent contamination or cross contamination of all the components. Also, avoid primer-dimer of PCR primers to eliminate false positive outcome.

Refer to Frequently Asked Questions at www.https//isobiotech.com.au for more information

5. Good laboratory practices for IPA

Adhere strictly to the following when preparing samples for IPA™-Green:

A. Wear clean gloves and a clean laboratory coat.
B. Do not wear the same gloves and laboratory coat used previously for handling amplified products or preparing samples. Change gloves if you suspect that they are contaminated.

C. Change your NTC or/and negative control or Master Mix if you suspect template contamination.
D. Maintain separate areas and dedicated equipment and supplies for sample preparation and reaction set-up, and amplification and analysis of amplified products.
E. Do not bring amplified products into the reaction set-up area.
F. Open and close all sample tubes carefully. Avoid splashing or spilling samples.
G. Keep reactions and components capped as much as possible.
H. Use a positive-displacement pipette or aerosol-resistant barrier pipette tips.
I. Clean laboratory benches and equipment periodically with freshly prepared 10% bleach solution or DNA decontamination solution.

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