Supplementary Protocol August 2017

Test Assay for Fast Reaction Mix 2.0

This protocol describes how to perform incoming QC on FRM 2.0, to confirm the absence of unspecific amplification on non-human DNA. The assay is designed to force unspecific amplification of FRM 2.0 lots, prior to the change in specification (Reference lot).

IMPORTANT: Please refer to the *Investigator® 24plex QS Kit Handbook* and the *Investigator 24plex GO! Kit Handbook* for general information on safety, handling and storage. Unspecific amplifications are unstable by nature and changes in reaction conditions may have an impact on absence or presence of individual peaks, and peak heights observed with affected FRM 2.0 reference lots. The assay requires a reference lot for side-to-side comparison. Only use the outer positions of the PCR cycler with labware that has been used with samples showing the issue in the past. If possible, do not change the PCR cycler or labware between assay runs to ensure more consistent results. If the assay fails to produce unspecific amplification with the reference lot, the annealing temperature can be lowered to further force the issue.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Investigator 24plex QS Kit (cat. nos. 382426 and 382428)
- Test DNA 1 supplied by QIAGEN R&D
- Pipets and pipet tips
- PCR tubes or plates
- Vortexer

Important points before starting

- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.

Things to do before starting

- Before opening the tubes containing PCR components, vortex and then centrifuge briefly to collect the contents at the bottom of the tubes.

Procedure

1. Thaw PCR components and Test DNA 1.

   Mix thoroughly. Centrifuge briefly before use.

2. Prepare a master mix according to Table 1 for the test and reference FRM 2.0 each.

   The master mix contains all of the components needed for PCR. Prepare a volume of reaction mix 10% greater than that required for the total number of PCR assays to be performed. We recommend running 4 replicates for the test and reference lot each in column 1 of the PCR plate. If tubes are used, place them on outer PCR block positions only. If more than 4 replicates are run, use column 12 in addition.

3. Program the thermal cycler according to the manufacturer's instructions, using the conditions given in Table 2.

   Note: If using the Applied Biosystems® GeneAmp® 9700 thermal cycler with an Aluminum block, use "Std Mode", or with a Silver block or Gold-plated Silver block, use "Max Mode". Do not use "9600 Emulation Mode".

4. After the completion of the protocol, add 1 µl of the PCR product directly to 12 µl HiDi™ Formamide plus the Size Standard. Start the analyzer run, as described in the corresponding kit handbook.

5. If the reference lot does not show amplification products, repeat the assay using a cycling protocol with lower annealing temperatures (Table 3).

Table 1. Master mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free water	5 µl
Test DNA 1	10 µl
Total volume	**25 µl**

Table 2. Recommended cycling protocol

Component	Time	Number of cycles
98°C*	30 s	3 cycles
64°C	55 s	
72°C	5 s	
96°C	10 s	27 cycles
61°C	55 s	
72°C	5 s	
68°C	2 min	
60°C	2 min	
10°C	∞	–

Table 3. Alternative cycling protocol

Component	Time	Number of cycles
98°C*	30 s	3 cycles
62°C	55 s	
72°C	5 s	
96°C	10 s	27 cycles
59°C	55 s	
72°C	5 s	
68°C	2 min	
60°C	2 min	
10°C	∞	–

Troubleshooting

For general troubleshooting, please consult the "Troubleshooting Guide" in the *Investigator 24plex QS Kit Handbook* and the *Investigator 24plex GO! Kit Handbook*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.