April 2021

Quick-Start Protocol

Investigator® 24plex GO! Kit, Part 2

Protocol for buccal cells from Bode Buccal DNA Collectors™

All components of the Investigator 24plex GO! Kit should be stored at –30 to –15°C. Avoid repeated thawing and freezing. The primer mix, allelic ladder, and DNA Size Standard must be stored protected from light. DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the kit.

Further information

- *Investigator 24plex GO! Handbook*: **www.qiagen.com/HB-1913**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes 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 with hydrophobic filters to minimize cross-contamination risks.
- Before opening the tubes, thaw PCR components, vortex, and then centrifuge briefly to collect the contents at the bottom of the tubes.
- For buccal cells on Bode Buccal DNA Collectors, Investigator STR GO! Lysis Buffer (QIAGEN, cat. no. 386516) must be ordered separately.

Procedure

1. Collect a 1.2 mm punch from the tip (rounded end) of the Bode Buccal DNA Collector with a suitable tool (e.g., UniCore Punch Kit 1.2 mm, cat. no. WB100028) into a 0.2 ml PCR-grade plate or 0.2 ml PCR-grade tube.

   Important: Do not use more than one punch at a time, per well or per tube.

2. Add 2 µl of Investigator STR GO! Kit Lysis Buffer directly onto the 1.2 mm punch. Centrifuge briefly if necessary to collect the punch and buffer at the bottom of the plate or tube.
3. Incubate the sample at 95°C for 5 min. Do not seal the plate.
4. Prepare a master mix according to Table 1. Vortex the reaction mix thoroughly.

   As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions. The master mix contains all of the components needed for PCR except the template (sample) DNA.

Table 1. Master mix setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	12.5 µl
Total volume	20.0 µl

5. After incubation, dispense 20 µl of the master mix into each well of the PCR plate or the PCR tubes, containing the 1.2 mm punch. Do not mix the reaction after distributing the master mix.
6. Prepare positive and negative controls.

   Positive control: Use 2 µl Control DNA (i.e., 10 ng).

   The amount of Control DNA may need to be adapted after setting the optimal PCR cycle number in your laboratory, if signals are too low or too high. Do not add a blank disc to the positive control well.

   Negative control: Do not add any template DNA. Do not add a blank disc or water to the negative control PCR tube or well.

7. Briefly centrifuge reactions to ensure discs are fully submerged.

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

   Note: If using the 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".

Table 2a. Standard cycling conditions

Temperature	Time	Number of cycles
98°C*	30 s	
64°C	40 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	40 s	24 cycles
72°C	5 s	
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Table 2b. Optional cycling conditions

Temperature	Time	Number of cycles
98°C*	30 s	
64°C	40 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	40 s	24 cycles
72°C	5 s	
68°C	2 min	–
60°C	2 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

Table 2b details previously published cycling conditions, which may continue to be used if incomplete adenylation is not visible within the electropherograms.

9. After the cycling protocol is completed, store samples at –30 to –15°C protected from light, or proceed directly with electrophoresis.

Document Revision History

Date	Changes
04/2021	This is the initial release of the second part of the Investigator 24plex GO! Kit Quick-Start Protocol, which is divided into 3 parts for printing purposes. The first and third parts of the Quick-Start Protocol are HB-1907-006 and HB-2895-001, respectively.

Scan QR code for handbook.

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