April 2021

Quick-Start Protocol

Investigator® 24plex GO! Kit, Part 1

Protocol for blood or buccal cells on FTA® or other paper

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 paper, Investigator STR GO! Punch Buffer (1000) or (200) (QIAGEN, cat. no. 386528 or 386526) must be ordered separately.

Procedure

1. Take a 1.2 mm punch from the center of the spot with a suitable tool (e.g., UniCore Punch Kit 1.2 mm, cat. no. WB100028).

   Note: For buccal cells collected using Indicating FTA Cards, take the punch from a white area. This color indicates successful sample transfer.

   Important: Do not use more than one punch at a time.

2. Prepare a master mix according to Table 1.

   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

	Volume per reaction	
Component	**Blood on FTA or other paper**	**Buccal cells on FTA or other paper**
Fast Reaction Mix 2.0	7.5 µl	7.5 µl
Primer Mix	12.5 µl	12.5 µl
Investigator STR GO! Punch Buffer	–	2.0 µl
Total volume	20.0 µl	22.0 µl

Note: In case blood on FTA samples has been stored for longer periods, we recommend the Investigator STR GO! Punch Buffer be used to overcome potential inhibition. Add 3 µl Investigator STR GO! Punch Buffer per reaction.

3. Vortex the reaction mix thoroughly, and dispense 20 µl for blood samples and 22 µl for buccal cell samples into PCR tubes or the wells of a PCR plate.
4. Transfer one 1.2 mm disc to each reaction.

   Note: Do not mix the reaction after disc transfer.

5. Prepare the positive and negative controls.

   Positive control: Use 2 µl Control DNA for blood or 1 µl Control DNA for buccal cells on FTA or other paper.

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

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

7. 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	Blood on FTA or other paper: 22 cycles Buccal cells on FTA or other paper: 23 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	Blood on FTA or other paper: 22 cycles Buccal cells on FTA or other paper: 23 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.

8. 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
02/2021	Table 2 is split into Tables 2a and 2b, Table 4 is split into Tables 4a and 4b, and Table 6 is split into Tables 6a and 6b. Editorial and styling changes.
04/2021	Divided the Quick-Start Protocol into 3 parts for printing purposes. The second and third parts of this Quick-Start Protocol are HB-2889-001 and HB-2895-001, respectively.

Scan QR code for handbook.

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