

Investigator[®] 24plex GO! Kit

Part 3: Protocol for buccal swabs

All components of the Investigator 24plex GO! Kit (cat. no. 382426 or 382428) should be stored at -30°C 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! Kit 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 the PCR components, vortex, and then centrifuge briefly to collect the contents at the bottom of the tubes.
- For the swab lysis protocol, Investigator STR GO! Lysis Buffer (QIAGEN, cat. no. 386516) must be ordered separately.

Procedure

1. Place the swab in a 2 mL microcentrifuge tube.
Carefully cut, break off, or eject the end part of the swab.
Note: Prepare a blank swab as negative control.
2. Add 500 μL STR GO! Lysis Buffer to the sample.
3. Incubate at 95°C for 5 min shaking at 1200 rpm in a thermomixer.

Optional: Incubate at room temperature for 5 min shaking at 1200 rpm in a thermomixer.

4. Prepare a master mix according to Table 1. The master mix contains all of the components needed for PCR except the template (sample) DNA.

As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also include positive and negative control reactions.

Table 1. Master mix setup

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	7.5
Primer Mix	12.5
Total volume	20.0

5. Vortex the reaction mix thoroughly and dispense 20 µL into the PCR tubes or the wells of a PCR plate.
6. Mix the swab lysate thoroughly and transfer 2 µL of swab lysate directly to each reaction.
7. Prepare positive and negative controls.

- **Positive control:** Use 1 µL Control DNA (i.e., 5 ng).

Note: 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:** Use a blank swab lysate.

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

Note: When using the GeneAmp PCR System 9700 with an Aluminum Block, select **Std Mode**; when using a Silver Block or Gold-plated Silver Block, select **Max Mode**. Do not select **9600 Emulation Mode**.

Note: For cyclers with higher ramp rates (e.g., VeritiPro Thermal Cycler), adjust the ramp rates to 4°C/s. Alternatively, if the cycler offers an emulation mode, enable it and select a cycler that is included in the validation report.

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

Table 2a. Standard cycling protocol for buccal swab lysates

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

* Hot-start to activate DNA polymerase.

Table 2b. Optional cycling protocol for buccal swab lysates

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

* Hot-start to activate DNA polymerase.

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

Document Revision History

Date	Description
04/2021	This is the initial release of the third part of the <i>Investigator 24plex GO! Kit Quick-Start Protocol</i> , which is divided into 3 parts for printing purposes. The first and second parts of the Quick-Start Protocol are HB-1907 and HB-2889, respectively.
05/2026	Added note on ramp rates for fast cyclers.



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