Quick-Start Protocol May 2026

Investigator® 24plex QS Kit

All components of the Investigator 24plex QS Kit 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 QS Kit Handbook*: **www.qiagen.com/HB-1860**
- 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 analysis).
- Use disposable tips containing 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.
- The recommended amount of DNA under standard conditions is 0.5 ng. Internal validations demonstrated robust and balanced results with 0.2–2 ng DNA and reliable results with <0.1 ng DNA.

Procedure

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

   As some loss of reagents can occur during transfers, prepare the mix with additional reactions included. Also, include positive and negative control reactions.
2. Vortex the reaction mix thoroughly, centrifuge briefly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.

3. Add template DNA and nuclease-free water to the master mix to yield a final sample volume of 25 µL.
4. Prepare the negative control (nuclease-free water) and positive control, which should be 5 µL of the Control DNA 9948 (0.1 ng/µL).

Table 1. Master mix setup

Component	Volume (µL) per reaction
Fast Reaction Mix 2.0	7.5
Primer Mix	2.5
Nuclease-free water (added in step 4)	variable
Template DNA (added in step 4)	variable
Total volume	**25.0**

5. 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® 9700 thermal cycler 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., VertiPro™ 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.
6. After the cycling protocol is completed, store samples at –30°C to –15°C protected from light, or proceed directly to running the electrophoresis.

Table 2a. Standard cycling protocol

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

* Hot-start to activate DNA polymerase.

Table 2b. Optional cycling protocol

Temperature (°C)	Time	Number of cycles
98*	30 s	
64	55 s	3
72	5 s	
96	10 s	
61	55 s	27
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 in the electropherograms.

Sample preparation for capillary electrophoresis

Before conducting DNA fragment size analysis, it is necessary to perform a spectral calibration for each analyzer with the six fluorescent labels: 6-FAM™, BTG, BTY, BTR2, BTP, and BTO. The calibration procedure creates a matrix that is used to correct for the overlapping of the dye fluorescence-emission spectra. Detailed protocols for using Applied Biosystems® 3500/3500XL Genetic Analyzers are provided in the *Investigator 24plex QS Kit Handbook*.

1. Set up a mixture of formamide and DNA Size Standard according to Table 3.
2. For each sample to be analyzed, aliquot 12 µL of the mixture to a tube.
3. Add 1 µL PCR product or allelic ladder (diluted, if necessary).
4. Denature for 3 min at 95°C.
5. Snap freeze by placing the plate on ice for 3 min. Alternatively, a thermal cycler set to 4°C may be used to cool the plate.
6. Load the samples on the Genetic Analyzer tray and start the run. Please check the *Investigator 24plex QS Kit Handbook* for detailed information on the run module settings (e.g., injection time, injection voltage, and run time).

After the run is finished, data can be analyzed using suitable software such as Applied Biosystems GeneMapper® *ID-X*. For more information, refer to the *Investigator 24plex QS Kit Handbook* and corresponding software user guides.

Table 3. Setup of formamide and DNA size standard mixture

Component	Volume (µL) per sample
Hi-Di™ Formamide	12.0
DNA Size Standard 24plex (BTO)	0.5

Document Revision History

Date	Description
02/2021	Table 2 is split into Tables 2a and 2b. Editorial and styling changes.
05/2026	Added note on ramp rates for fast cyclers. Corrected size standard information in Table 3.

Scan the QR code for handbook.

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