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Quick-Start Protocol

Investigator® Argus Y-28 QS Kit

All components of the Investigator Argus Y-28 QS Kit (cat. nos. 383625 and 383627) 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 PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the kit.

Further information

- *Investigator Argus Y-28 QS Handbook*: **www.qiagen.com/HB-2897**

  Please note that the handbook contains additional protocols, e.g., for amplification of low amounts of template, for direct amplification of various reference sample materials, and for reduced reaction volumes.
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Set up all the 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.
- The recommended amount of DNA under standard conditions is 0.5 ng.

Procedure

1. Prepare 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 and nuclease-free water.

Table 1. Reaction setup

Component	Volume per reaction
Fast reaction mix 3.0	7.5 µl
Primer mix	2.5 µl
Nuclease-free water	Variable
Template DNA	Variable
Total volume	25 µl

2. Vortex thoroughly and dispense appropriate volumes of Master Mix into PCR tubes or the wells of a PCR plate.
3. Add template DNA and nuclease-free water to give 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).

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

Table 2. Cycling conditions

Temperature	Time	Number of cycles
96°C*	12 min	–
96°C 61.5°C 72°C	10 s 1 min 25 s 5 s	30 cycles
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

* Hot-start to activate DNA polymerase.

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

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 the Applied Biosystems® 3500/3500xL Genetic Analyzers are provided in the *Investigator Argus Y-28 QS Handbook*.

1. Set up a mixture of formamide and DNA size standard according to Table 3.

Table 3. Setup of formamide and DNA size standard mixture

Component	Volume per sample
Hi-Di™ Formamide	12.0 µl
DNA size standard BTO	0.5 µl

2. For each sample to be analyzed, aliquot 12 µl of the mixture to a tube.
3. Add to the mixture 1 µl PCR product or allelic ladder (diluted, if necessary).
4. Denature the plate for 3 min at 95°C.
5. Snap freeze the plate by placing it 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 refer to the *Investigator Argus Y-28 QS Handbook* for detailed information on the run module settings (e.g., injection time, injection voltage, and run time).
7. After the run is finished, data can be analyzed using suitable software, such as the Applied Biosystems GeneMapper® ID-X Software. For more information, refer to the *Investigator Argus Y-28 QS Handbook* and the corresponding software user guides.

Document Revision History

Date	Changes
05/2021	Initial release

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.