Investigator® ESSplex SE QS Kit

All Investigator ESSplex SE QS Kit (cat. nos. 381575, 381577) components should be stored at –30 to –15°C. Avoid repeated freeze/thawing. The Primer Mix, Allelic Ladder, and DNA Size Standard 550 (BTO) should be stored in a dark place. DNA samples and post-PCR reagents (Allelic Ladder and DNA Size Standard 550 [BTO]) should be stored separately from the PCR reagents. Under these conditions, the kit components are stable until the expiration date indicated on the box label.

Further information

- *Investigator ESSplex SE QS Kit Handbook*: **www.qiagen.com/HB-1963**
- 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 risk.
- Before opening the tubes, thaw PCR components, vortex, and 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. Using <0.1 ng DNA provides reliable results, although stochastic effects may increase with low amounts of DNA.

PCR amplification procedure

1. Prepare master mix according to Table 1.

   Note: Prepare excess master mix, as loss of reagents can occur during transfer. The master mix contains all the required PCR components,

besides the template (sample) DNA and Nuclease-free Water. Always include positive and negative controls in your PCR run.

Table 1. Reaction setup

Component	Volume per reaction
Fast Reaction Mix 2.0	7.5 µl
Primer Mix	2.5 µl
Nuclease-free Water (added in step 4)	Variable
Template DNA (added in step 4)	Variable
Total volume	25 µl

2. Vortex thoroughly and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
3. Add template DNA and Nuclease-free Water to reach a final sample volume of 25 µl.
4. Prepare negative (Nuclease-free Water) and positive controls (5 µl control DNA 9948 [0.1ng/µl]).
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 2.

   Note: If using the GeneAmp PCR System 9700 with an Aluminum Block, use "Std Mode"; with a 96-Well Silver Sample Block or 96-Well Gold-Plated Silver Sample Block, use "Max Mode". Do not use "9600 Emulation Mode".

Table 2a. Standard cycling conditions

Component	Time	Number of cycles
98°C*	30 s	
64°C	55 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	55 s	27 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

Component	Time	Number of cycles
98°C*	30 s	
64°C	55 s	3 cycles
72°C	5 s	
96°C	10 s	
61°C	55 s	27 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.

6. Once the cycling protocol is complete, store samples at –30 to –15°C in the dark or proceed directly with electrophoresis.

Capillary electrophoresis procedure

Before conducting DNA fragment size analysis, it is necessary to perform a spectral calibration for each analyzer with the five fluorescent labels: 6-FAM™, BTG, BTY, BTR, and BTO. Detailed protocols for commonly used Applied Biosystems Genetic Analyzers are provided in the *Investigator ESSplex SE QS Kit Handbook*, which can be found at **www.qiagen.com/handbooks.**

1. Set up a mixture of formamide and DNA Size Standard 550 (BTO) according to Table 3.

Table 3. Formamide and DNA Size Standard mixture setup

Component	Volume per sample
Hi-Di™ Formamide	12.0 µl
DNA Size Standard 550 (BTO)	0.5 µl

2. For each sample to be analyzed, aliquot 12 µl of the mixture into a PCR tube.
3. Add 1 µl PCR product or Allelic Ladder.

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 Applied Biosystems Genetic Analyzer tray and start the run with the run module settings provided in Table 4.

   Note: If you are using the Investigator ESSplex SE QS Kit for the first time, you will first need to set up a number of protocols, including the Instrument, Size Standard, QC, and Assay Setup Protocols. Check the *Investigator ESSplex SE QS Kit Handbook* for detailed information on the recommended settings for the respective CE instrument.
7. Once the CE run is complete, data can be analyzed using suitable software, such as the Applied Biosystems GeneMapper® *ID-X* Software. For more information, refer to the *Investigator ESSplex SE QS Handbook* and corresponding software user guides.

Table 4. CE injection conditions using POP4 Polymer and 36 cm Array Length

Genetic analyzer	Injection voltage	Injection time	Run voltage	Run time
ABI PRISM® 3100-*Avant*™/3100 Genetic Analyzer	2.5 kV	30 s	13 kV	1800 s
Applied Biosystems 3130/3130xL Genetic Analyzer	2.5 kV	30 s	13 kV	1800 s
Applied Biosystems 3500™ Genetic Analyzer	1.2 kV	30 s	13 kV	1550 s
Applied Biosystems 3500xL Genetic Analyzer	1.6 kV	25 s	13 kV	1550 s

Document Revision History

Date	Changes
02/2021	Split Table 2 into Tables 2a and 2b. Editorial and layout changes.

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