

# Investigator<sup>®</sup> ESSplex Plus Kit

All components of the Investigator ESSplex Plus Kit should be stored at  $-30$  to  $-15^{\circ}\text{C}$ . Avoid repeated thawing and freezing. The Primer Mix, allelic ladder and DNA size standard must be stored protected from the 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 ESSplex Plus Handbook*: [www.qiagen.com/HB-1153](http://www.qiagen.com/HB-1153)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://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.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.
- Before opening the tubes with PCR components, vortex them and then centrifuge briefly to collect 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.

1. Thaw PCR components and template nucleic acid.
2. Prepare a master mix according to Table 1.  
Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.
3. Mix the master mix thoroughly and dispense the appropriate volumes into PCR tubes or the wells of a PCR plate.
4. Add template DNA and nuclease-free water to the master mix, to give a final sample volume of 25  $\mu$ l.
5. Prepare positive and negative controls.

**Positive control:** Use 5  $\mu$ l Control DNA 9948 per reaction.

**Negative control:** Use nuclease-free water instead of template DNA.

6. For optimal results, mix the prepared and sealed PCR plate prior to amplification using an Eppendorf Thermomixer Comfort (orbital shaker) for 5 min at 1200 rpm at room temperature. Use constant shaking without pauses.
7. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 2.

**Note:** If using the GeneAmp® 9700 thermal cycler with an Aluminum block, use **Std Mode**. With a Silver block or Gold-plated Silver block, use **Max Mode**. Do not use **9600 Emulation Mode**.

**Table 1. Reaction setup**

Component	Volume per reaction
Fast Reaction Mix	7.5 $\mu$ l
Primer Mix ESSplex Plus	2.5 $\mu$ l
Nuclease-free water (added in step 4)	Variable
Template DNA (added in step 4)	Variable
<b>Total volume</b>	<b>25 <math>\mu</math>l</b>

**Table 2. Standard cycling protocol**

Temperature	Time	Number of cycles
95°C	5 min	–
96°C	10 s	30 cycles
61°C	120 s	
10°C	∞	–

8. After the cycling protocol is completed, store samples at –20°C protected from the 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 five fluorescent labels: 6-FAM™, BTG, BTY, BTR 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 commonly used Applied Biosystems® Genetic Analyzers are provided in the *Investigator ESSplex Plus Handbook*, which can be found at [www.qiagen.com/HB-1153](http://www.qiagen.com/HB-1153).

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 550 (BTO)	0.5 µl

2. Aliquot 12 µl of the mixture to a tube for each sample to be analyzed.
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, the thermal cycler set to 4°C may be used to cool the plate.

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6. Load the samples on the Genetic Analyzer tray and start the run.

Please check the *Investigator ESSplex Plus 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 QIAGEN® Investigator IDproof Software or IDproof Mixture Software, or Applied Biosystems GeneMapper® *ID-X* Software. For more information, refer to the *Investigator ESSplex Plus Handbook* and corresponding software user guides.



Scan QR code for handbook.

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

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