Quick-Start Protocol February 2015

Investigator® ESSplex SE GO! Kit

All components of the Investigator ESSplex SE GO! Kit (cat. nos. 381566, 381568) should be stored at –30 to –15°C. Avoid repeated thawing and freezing. 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 SE GO! Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

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 containing hydrophobic filters to minimize cross-contamination risks.
- Before opening the tubes with PCR components, vortex and then centrifuge briefly to collect contents at the bottom of the tube.
- To use the swab lysis protocol, the Investigator STR GO! Lysis Buffer (QIAGEN, cat. no. 386516) must be ordered separately
- For buccal cells on paper, Investigator STR GO! Punch Buffer (1000) or (200) (cat. no. 386528 or 386526) must be ordered separately.

Procedure for blood or buccal cells on FTA® or other paper

1. Take a 1.2 mm punch from the center of the spot with a suitable tool.

   Note: If using a Whatman™ EasiCollect™ and a buccal cell sample, take the punch from a white area. This color indicates successful sample transfer.

   IMPORTANT: Do not use more than one punch at a time.

2. Prepare a 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.

Table 1. Master Mix setup

	Volume per reaction		
Component	**Blood on FTA or other paper**	**Buccal cells on FTA or other paper**	**Buccal swabs**
Fast Reaction Mix	7.5 µl	7.5 µl	7.5 µl
Primer Mix	17.5 µl	17.5 µl	17.5 µl
Investigator STR GO! Punch Buffer	–	2.0 µl	–
Total volume	**25 µl**	**27 µl**	**25 µl**

3. Mix the reaction mix thoroughly, and dispense 25 µl (Blood on FTA) or 27 µl (Buccal cells on FTA) into PCR tubes or the well of a PCR plate.

4. Transfer one 1.2 mm disc to each reaction.

   Note: Do not mix the reaction after disc transfer.

5. Prepare the negative control (a blank disc) and positive control, which should be 2 µl Control DNA 9948 (5 ng/µl) for blood or 1 µl Control DNA for buccal cells.

6. Briefly centrifuge reactions to ensure discs are fully submerged.

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

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

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

Table 2. Cycling conditions

Component	Time	Number of cycles
95°C*	8 min	–
96°C	10 s	Blood on FTA or other paper: 25
61°C	38 s	Buccal cells on FTA or other paper: 28 Buccal swab lysates:28
68°C	1 min	–
10°C	∞	–

* Hotstart to activate DNA polymerase.

Procedure for buccal swab lysates

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 room temperature (15–25°C) for 5 min, shaking at 1200 rpm in a thermomixer.

   Optional: For challenging samples, results may be improved by incubating at 95°C in a thermomixer shaking at 1200 rpm.

4. Prepare a 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.

5. Mix the reaction mix thoroughly, and dispense 25 µl into a PCR tube or the well of a PCR plate.
6. Transfer 2 µl swab lysate to each reaction.
7. Prepare the positive and negative controls.

   Positive control: Use 1 µl Control DNA.

   Negative control: Use a blank swab lysate.
8. Program the thermal cycler according to the manufacturers' instructions, using the conditions given in Table 2.

   Note: If using the GeneAmp PCR System 9700 with an Aluminum Block, use "Std Mode", or with a Silver 96-Well Block or Gold-plated Silver 96 Well Block, use "Max Mode". Do not use "9600 Emulation Mode".
9. After the cycling protocol is completed, store samples at –30 to –15°C protected from the light, or proceed directly with electrophoresis.

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