Supplementary Protocol July 2022

Investigator® Casework GO! Kit using the Investigator Lyse&Spin Basket Kit

This protocol describes how to use the Investigator Casework GO! Kit (cat. no. 386546) using the Investigator Lyse&Spin Basket Kit (cat. nos. 19597 and 19598).

For use of Investigator Casework GO! with the Investigator Lyse&Spin Kit, modifications from *Investigator Casework GO! Kit Quick Start Protocol* (HB-2688) is required at the heated incubation stage because the temperature transfer to the sample might decrease due to the presence of two layers of plastic material.

Please note that Investigator Lyse&Spin baskets are not recommended for the differential fraction separation of sexual assault samples. The baskets are not designed for a complete flow-through of sperm cells, leading to a loss of these cells in the collected lysate. However, it can be used for sperm pellet lysates.

Storage

The Investigator Casework GO! Kit should be stored at 2–8°C. For semen samples only (to be ordered separately): 1M DTT should be stored at –30 to –15°C. After the first thawing, DTT should be stored in smaller aliquots at –30 to –15°C to avoid repeated freezing/thawing. If stored properly, the components are stable until the expiration date indicated on the kit.

Further information

- Investigator Casework GO! Kit product page: **www.qiagen.com/casework-go**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Perform sample preparation in an area that is separate from the one used for PCR assay setup and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination risks.
- Before opening the tubes with kit components, vortex, and then centrifuge briefly to collect contents at the bottom of the tube.
- Equipment: pipettes, vortex mixer, heat shaker; for semen: 1 M DTT, 1 ml, forensic grade quality (cat. no. 1117316)
- The following collection devices are recommended for the Investigator Casework GO! Kit:
  - Cotton swabs:
    - Puritan® sterile cotton-tipped applicators (Puritan cat. no. 25-806 1PC)
    - Heinz Herenz ETO-treated DNA-examinated swabs (Heinz Herenz cat no. 1020055)
    - Sarstedt® swab or forensic swab (Sarstedt cat. nos. 80.629 and 80.626, respectively)
  - Polyester swabs:
    - Puritan sterile polyester-tipped applicators (Puritan cat. no. 25-806 1PD)
    - Pushoff™ swab, standard bud (abf cat. no. 08228)
  - Flock swabs:
    - FLOQSwabs™ (Copan cat. nos. 552C and 502CS01) or microFLOQ® direct (Copan cat. no. 60U001D)
    - MicroTest™ (Thermo Fisher cat. no. R12542)
- Lower yields might occur with Crime Scene 4N6FLOQSwabs™ (e.g., Copan cat. no. 3503C)

Master Mix preparation for semen samples

1. Dilute 1 M DTT according to Table 1 to obtain a 10 mM DTT solution.

Table 1. Dilution of DTT (1:100)

Component	Volume
Nuclease-free water	495 µl
DTT, 1 M	5 µl
Total volume	**500 µl**

2. Vortex the diluted DTT thoroughly.
3. Depending on the size of the collection device or cutting, a lysis volume of 200–400 µl can be selected. Prepare fresh Master Mix according to Table 2 for the number of samples you like to process, plus a 10% excess (e.g., if you have 20 samples, prepare Master Mix for 22).

   Note: The Investigator Lyse&Spin Basket requires force provided by the sample and lysis buffer to open up upon centrifugation. If the lysate does not fully pass, increase the lysis buffer volume to 400 µl, or increase the centrifugation speed up to 20.000 x g.

Table 2. Setup of Master Mix for semen samples

Component	Volume per reaction	Volume per reaction	Volume per reaction
Casework GO! Lysis Buffer	187 µl	280.5 µl	374 µl
Proteinase K	7 µl	10.5 µl	14 µl
DTT, diluted 1:100	6 µl	9 µl	12 µl
Total volume	**200 µl**	**300 µl**	**400 µl**

4. Vortex the Master Mix thoroughly, and then briefly centrifuge. Continue with sample lysis.

Master Mix preparation for casework samples except semen

1. Depending on the size of the collection device or cutting, a lysis volume of 200–400 µl can be selected. Prepare fresh Master Mix according to Table 3 for the number of samples you like to process, plus 10% in excess (e.g., if you have 20 samples, prepare Master Mix for 22).

   Note: The Investigator Lyse&Spin Basket requires force provided by the sample and lysis buffer to open up upon centrifugation. If the lysate does not fully pass, increase the lysis buffer volume to 400 µl, or increase the centrifugation speed up to 20.000 x g.

Table 3. Setup of Master Mix for casework samples except semen

Component	Volume per reaction	Volume per reaction	Volume per reaction
Casework GO! Lysis Buffer	193 µl	289.5 µl	386 µl
Proteinase K	7 µl	10.5 µl	14 µl
Total volume	**200 µl**	**300 µl**	**400 µl**

2. Vortex the Master Mix thoroughly and then briefly centrifuge. Continue with sample lysis.

Sample lysis

1. Preheat a heater shaker to 65°C. (Ideally, also preheat a second heater shaker to 85°C.)
2. Place the stained material in the Investigator Lyse&Spin basket within a 2 ml microcentrifuge tube (provided). Add 200–400 µl Master Mix to your samples according to the size of the collection device or size of cutting, etc.
3. Place the sample tubes onto the heater shaker that has been preheated to 65°C, and incubate for 25 min with 900 rpm.
4. Centrifuge for 1 min at a minimum of 10,000 x g.

   Note: Keep the lid closed during centrifugation.

   Note: Up to 20,000 x g can be used for centrifugation.

   Note: Ensure that no liquid remains in the basket after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane. If larger pieces of chewing gum are processed, press the chewing gum against the sides of the basket to avoid clogging.
5. Discard the basket including the solid sample substrate.
6. Transfer the sample tubes onto a heater shaker that has been preheated to 85°C, and then incubate for 5 min without shaking.

   Note: This step improves lysis and inactivates the proteinase K.

   Important: It is critical that the lysate is through the basket before 85°C as this temperature can affect the valves in the basket and prevent liquid from transferring.
7. Set up PCR reactions or store samples at 2–8°C for storage up to 1 year, or at –30 to –15°C for storage periods >1 year.

   Note: Casework GO! Samples are compatible with all Investigator DNA quantification and STR kits.

Document Revision History

Date	Changes
07/2022	Initial release

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