

Investigator[®] Quantiplex[®] Pro RGQ Kit

Highest sensitivity and male DNA quality assessment for your forensic samples

The Investigator Quantiplex Pro RGQ Kit quantifies while simultaneously providing a qualitative assessment of both the human and male DNA in a sample using quantitative real-time PCR, based on TaqMan[®] technology and QIAGEN's fast reaction chemistry. An internal PCR control (IPC) is incorporated to provide a reliable and consistent inhibition marker, identifying PCR inhibitors that may interfere with downstream processes, such as STR PCR. In addition, DNA degradation can be accurately assessed separately for both the human and male DNA, indicating whether it may impair downstream STR analysis. The kit works with any human ID workflow, regardless of the upstream sample prep or downstream STR kit used, and is designed to complement the Quality Sensor included in several of our Investigator STR PCR assays (e.g., Investigator ESSplex SE QS Kit, Investigator 24plex QS Kits, etc.), enabling an unparalleled level of workflow optimization. The qualitative assessment of male DNA in a background of female DNA makes it ideally suited for use in sexual assault investigations.

- Highly accurate and sensitive results for male DNA, even in a high background of female DNA (up to 1,000,000:1)
- Precise and independent assessment of DNA degradation for both male and total human DNA
- High dynamic range from 0.5 pg/ μ l to 200 ng/ μ l for both human and male DNA
- Reliable information about inhibitors with high correlation to STR results
- Fast-cycling technology and rapid quantification in about one hour

Partners against crime

Quantification is only one part of a complex forensic workflow where all steps need to be optimized and aligned to obtain the best possible results from your samples. This is why we have developed the Investigator Quantiplex Pro RGQ Kit to help you achieve the most meaningful data in the context of downstream STR PCR. The alignment of quantification and STR PCRs is achieved using the IPC present in our Quantiplex Pro Kits and the Quality Sensor included in the Investigator STR PCR Kits (Figure 1). [▶](#)

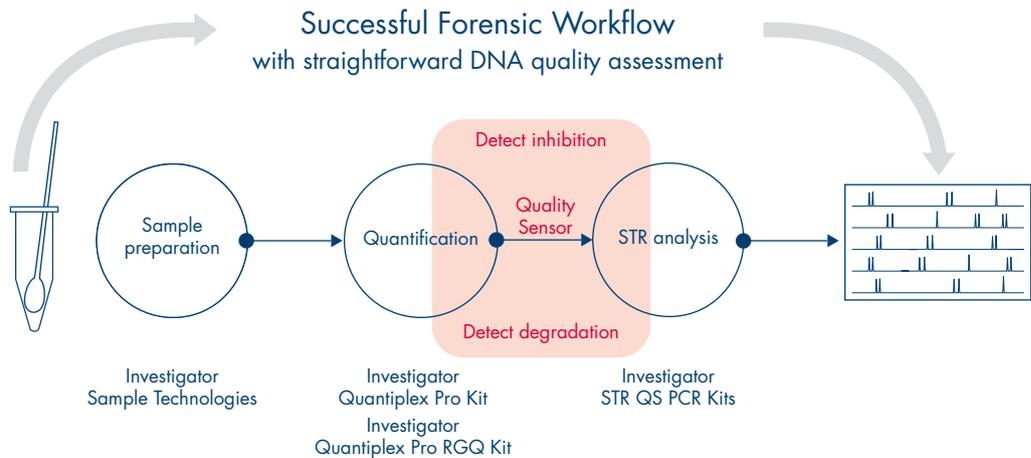


Figure 1. The Investigator Quantiplex Pro RGQ Kit provides information on inhibition and degradation, as well as accurate human and male DNA quantification relevant to downstream STR PCR. When STR kits containing the Quality Sensor are used in combination with the Quantiplex Pro Kits, workflow synergy is maximized since quantification and STR PCRs work together to predict and confirm PCR performance.

Because the IPC in the Quantiplex Pro RGQ is designed to behave similarly to the Quality Sensor in the presence of inhibitors, the two different PCR assays complement each other to predict and confirm the performance of the STR PCR, allowing you to interpret your results with maximum confidence.

Degradation (Human Degradation Index, HDI and Male Degradation Index, MDI)

Regardless of the presence of a high concentration of DNA in your sample, your STR PCR is unlikely to be successful if the DNA is severely degraded. In order to save valuable time, reagents and costs, and to enable you to choose the right downstream analysis method for your samples, the Investigator Quantiplex Pro RGQ Kit includes small and large male and human DNA targets to provide an accurate assessment of DNA degradation. The male and human ‘large’ targets are both over 350 bp in size, enabling the maximum possible insight into the quality of high-molecular-weight loci in the STR profile of a sample containing DNA from both male and female contributors. Moreover, because the small and large targets are so different in size, the effect of degradation on their respective amplification is significantly different (Figure 2). The relative amplification of these male and human ‘small’ and ‘large’ fragments is a powerful tool for predicting DNA degradation in both male and total human DNA samples, and this can be directly correlated to the likely performance of your STR PCR. This is particularly beneficial when analyzing sexual assault samples, where low levels of the male perpetrator’s DNA could be highly degraded in a

background of non-degraded female DNA. This unique feature therefore enables an accurate qualitative assessment of the male DNA in such samples, allowing the precise determination of subsequent processing steps (for instance, Y-STRs or even Y-SNPs for highly degraded male DNA).

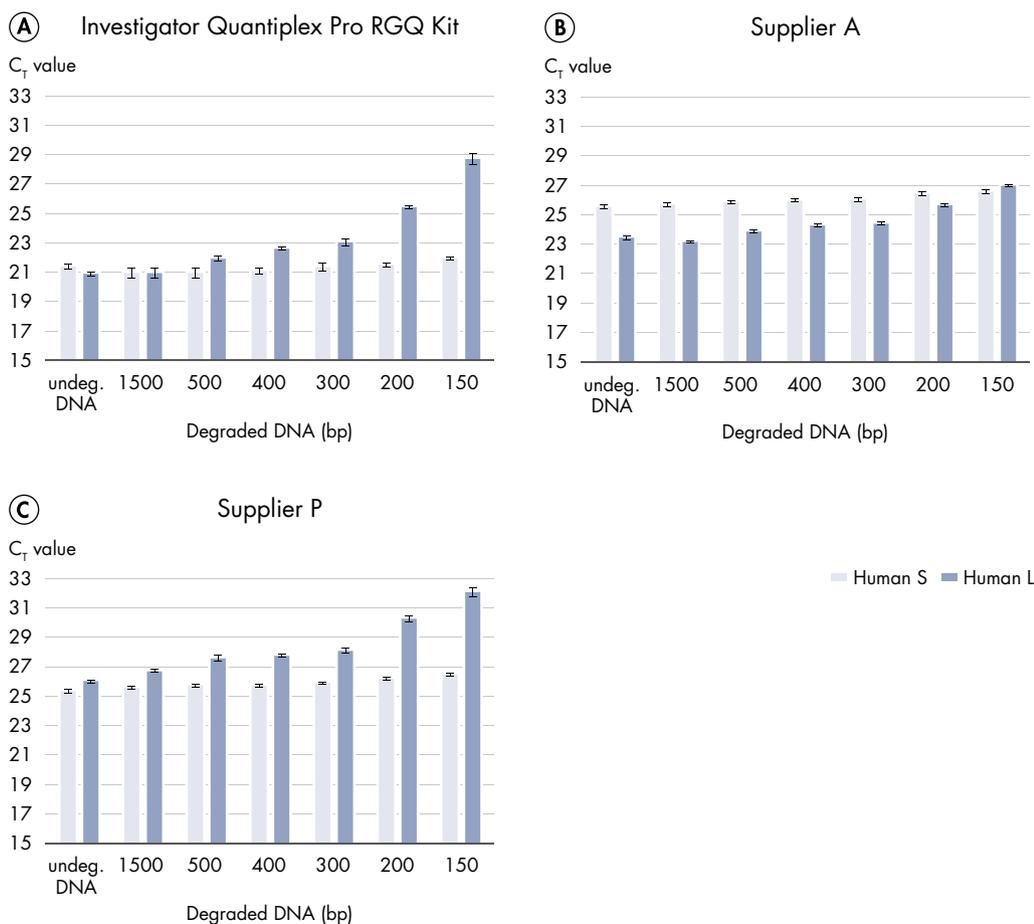


Figure 2. Accurate detection of DNA degradation. Male genomic DNA was sheared with a Covaris® S220 Focused-ultrasonicator™ to average fragment sizes of 1500 bp, 500 bp, 400 bp, 300 bp, 200 bp, and 150 bp. **A** Each fragment size (4.6 ng) was tested using the Investigator Quantiplex Pro RGQ Kit. The performance of two other commercially available kits under the same test conditions is shown in **B** and **C**. All reactions were set up and run according to the manufacturer’s instructions. There was a higher shift in C_t value for the QIAGEN human L fragment compared to kits from alternative suppliers, indicating a more accurate assessment of DNA quality in the sample.

The Investigator Quantiplex Pro RGQ Kit is highly efficient in detecting male DNA degradation, even in a high background of female DNA, in comparison to competitor kits (Figure 3). Accurate and reproducible quantification of degraded male DNA enables precise determination of downstream analysis steps, such as the use of a Y-STR Kit with increased DNA input volume.



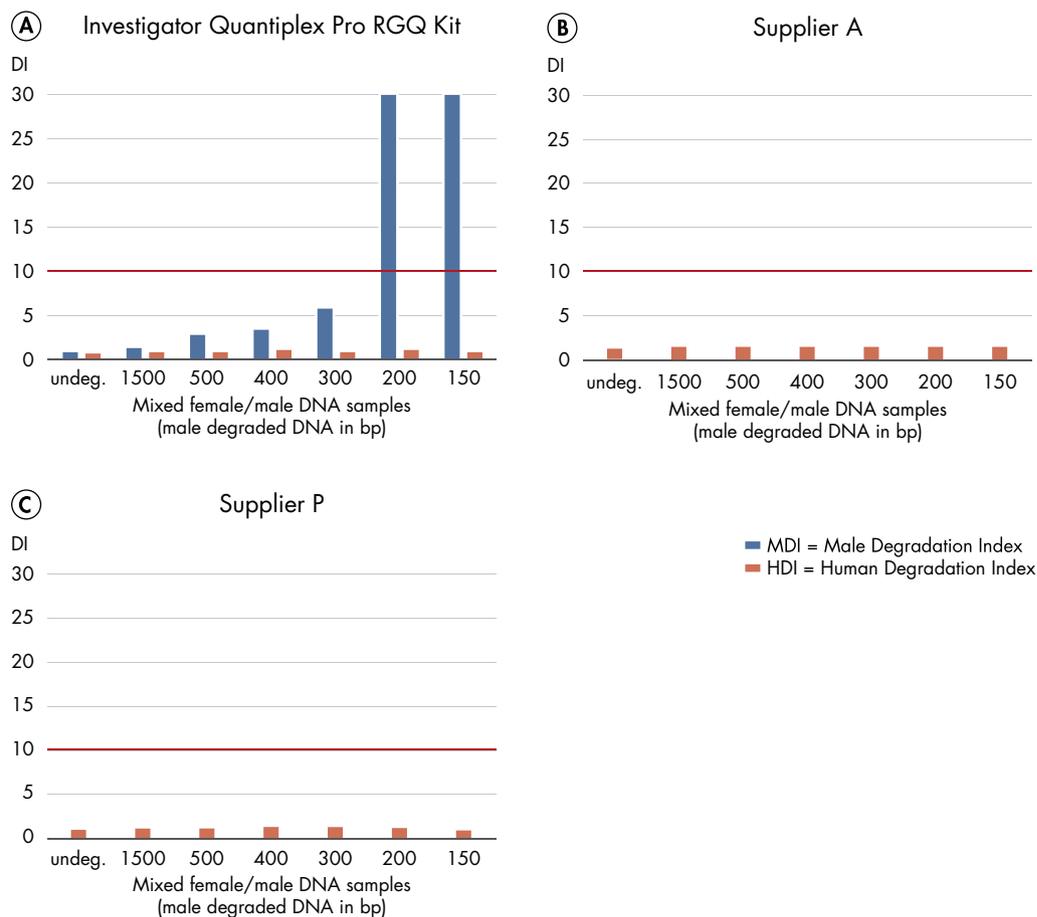


Figure 3. Accurate detection of male DNA degradation in a sample mixture. Male genomic DNA was sheared with a Covaris S220 Focused-ultrasonicator to average fragment sizes of 1500 bp, 500 bp, 400 bp, 300 bp, 200 bp, and 150 bp. Each set contains 0.1 ng/ μ l of degraded male DNA mixed with 100 ng/ μ l of non-degraded female DNA. Each sample set was tested using the Investigator Quantiplex Pro RGQ Kit (A) and competitor kits (B and C). While the Investigator Quantiplex Pro RGQ Kit could accurately detect the degradation status of the male DNA in the sample mixture with a high female DNA background, competitor kits failed to detect male DNA degradation.

In a crime sample workflow, samples identified as containing low levels of degraded male DNA in a background of excess, non-degraded female DNA would not be expected to give useful male DNA profiles unless a Y-STR kit was used to target the male DNA. To demonstrate this workflow, and to show the correlation of the male degradation result with a useful Y-STR profile, the Investigator Argus Y-12 QS Kit was used for detecting the male DNA profile from mixtures of male and female DNA. The kit enables simultaneous amplification of 12 Y-chromosomal STR loci in a single PCR (Figure 4).

Amplification of degraded male DNA in high female background with ArgusY

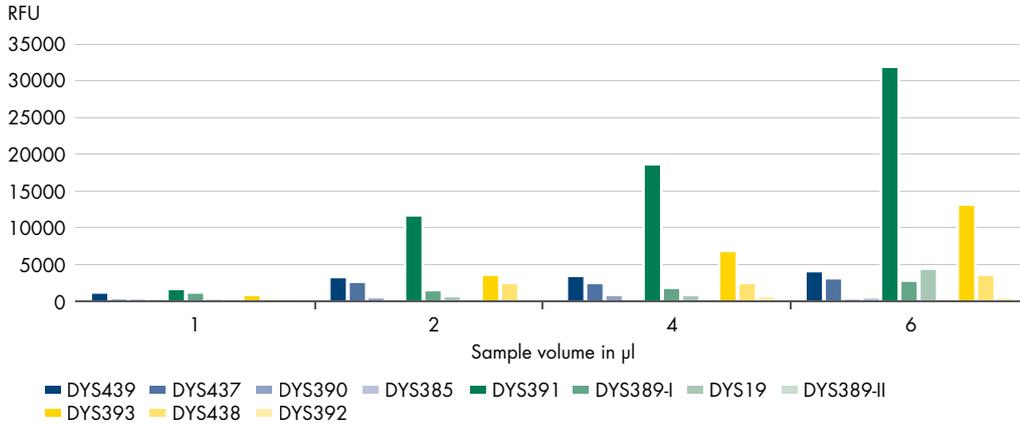


Figure 4. Male DNA profiling using the Investigator Argus Y-12 QS Kit. Samples from figure 3 with male DNA degraded to 150 bp and mixed with non-degraded female DNA were amplified with the Investigator Argus Y-12 QS Kit. These data demonstrate that male DNA degradation can be compensated by increasing the sample input volume to obtain a useful STR profile.

Sensitivity and dynamic range

Forensic and human identity samples are notoriously challenging to work with because of varying levels of DNA, from extremely high (e.g., visible blood stains) to traces (e.g., single cells or cell-free DNA). Furthermore, in many sexual assault samples, the quantity of male DNA in a much larger background of female DNA needs to be accurately determined. For these reasons, the Investigator Quantiplex Pro RGQ Kit has been developed with reliable, multi-copy male and human DNA targets to provide unparalleled sensitivity and dynamic range. These targets enable accurate and reproducible quantification of both male and human DNA, regardless of the amount of input DNA used (Figure 5).

High sensitivity for male DNA

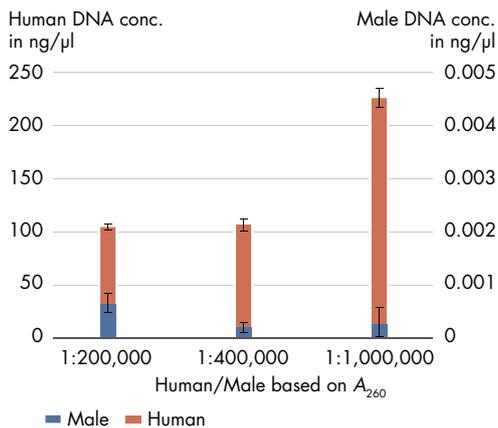


Figure 5. Highly accurate and sensitive detection results for male and total human DNA. Three different mixture sets were tested in duplicate (0.5 pg/µl male DNA + 100 ng/µl female DNA (1:200,000), 0.25 pg/µl male DNA + 100 ng/µl female DNA (1:400,000), and 0.2 pg/µl male DNA + 200 ng/µl female DNA (1:1,000,000)). The Investigator Quantiplex Pro RGQ Kit accurately quantified the male and female DNA.

Reliable detection of PCR inhibition

Forensic samples can be delivered on any matrix depending on the case circumstances, and therefore, they frequently contain substances deleterious to successful DNA profiling. Even with the most robust sample prep method, some of these substances are still present in the purified DNA and will inhibit the downstream STR PCR, unless identified and corrected for. The Investigator Quantiplex Pro RGQ Kit includes an IPC that is both sensitive to PCR inhibitors and responds to inhibition in a highly linear fashion (Figure 6). In this way, the IPC not only identifies inhibition, at a sensitivity closely matched to that of STR assays, but also provides accurate quantitative feedback on inhibitor levels that can be used as a basis for choosing the most appropriate downstream analysis method for your samples.

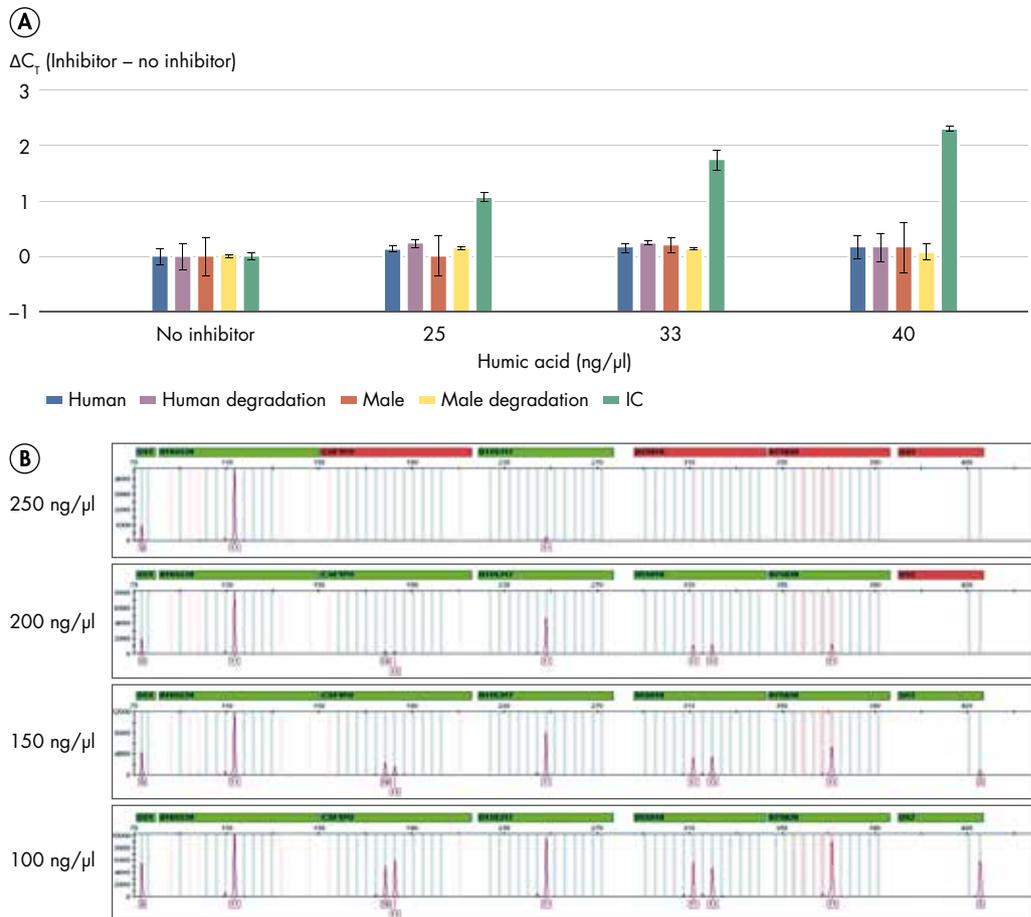


Figure 6. Identification of PCR inhibition. **A** The Investigator Quantiplex Pro RGQ Kit was run in the presence of 0, 25, 33, and 40 ng/μl humic acid on the Rotor-Gene® Q real-time PCR system. The internal control (in green) acts as Quality Sensor and reports the presence of the inhibitor with a C_t shift, while quantification (in blue and red for human and male DNA) remains reliable up to a final humic acid concentration of 40 ng/μl. **B** The Investigator 24plex QS Kit shows resistance to humic acid of up to 200 ng/μl (based on the final concentration in the STR reaction), while the Quality Sensor in the Investigator 24plex Kit (the far right homozygous locus of each line) reports the presence of inhibitors with the same level of sensitivity as the Investigator Quantiplex Pro RGQ Kit.

Technical specifications

Parameter	Investigator Quantiplex Pro RGQ Kit
Human target, small (length/channel)	91 bp/yellow channel
Human target, large (length/channel)	353 bp/red channel
Human male target, small (length/channel)	81 bp/green channel
Human male target, large (length/channel)	359 bp/orange channel
Internal PCR Control (length/channel)	434 bp/crimson channel
Volume per reaction	20 µl
PCR speed on the RGQ	~1 hour

Ordering Information

Product	Contents	Cat. no.
Investigator Quantiplex Pro RGQ Kit (200)	Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
QIAgility System HEPA/UV (incl. PC)	Robotic workstation for automated PCR setup (with UV light and HEPA filter); including notebook computer, QIAgility Software, installation and training, 1-year warranty on parts and labor	9001532
Rotor-Gene Q 6plex HRM System	Real-time PCR cycler with 6 channels (blue, green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001660

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Learn more about our quantification solutions for human identity and forensic testing at www.qiagen.com/quantiplexkits.

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