

INTERNAL VALIDATION OF QUANTIFILER® DUO DNA QUANTIFICATION KIT

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The Quantifiler® Duo DNA Quantification Kit, which provides estimates of both total human and male DNA simultaneously in forensic samples, was validated on Applied Biosystems 7500 Real Time PCR Systems. Preparation of the 96-well plates was performed on Tecan® Freedom EVO® workstations. Reaction volumes were maintained at 25 µl, as recommended by the manufacturer but standard DNA concentrations were cut in half and the most dilute standard (0.023 ng) eliminated. We demonstrate satisfactory reproducibility, repeatability, precision, sensitivity and reliable estimation of male DNA quantities even in the presence of 5000-fold excess female DNA. Representative forensic samples were tested from different biological sources extracted from various substrates. Good correlations were also observed between DNA estimates with this kit and STR profiles obtained using ProfilerPlus® Amplification kits.

The broad range of linear response of the Quantifiler® Duo Kit has allowed assaying a single sample from each forensic DNA extract. Concentrated samples (e.g. references, blood) are diluted 10-fold before quantification while low DNA-containing samples are assayed as is. Epithelial fractions from female sexual assault victims, which usually contain large amounts of the victim's DNA, are assayed both 10-fold diluted to quantify the female DNA and undiluted, to ensure detection of the male DNA in cases where it is present in low levels.