

ALUQUANT™ HUMAN DNA QUANTITATION SYSTEM IN THE FLUOROSCAN ASCENT FL LUMINOMETER

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The AluQuant™ Human DNA Quantitation System uses DNA probes that are specific to repetitive human elements allowing quantitation without PCR amplification. Quantitation of human DNA by the AluQuant™ Human DNA Quantitation System is provided by a series of reactions. Following an initial denaturation, DNA samples are incubated with the READase™ Polymerase, READase™ Kinase and human-specific probe. This coupled enzymatic reaction produces ATP relative to the amount of human DNA present. In a second incubation, ATP produced in the first reaction is used by Luciferase to produce a proportional and measurable amount of light. Background noise is determined by concurrent analysis of the sample without the human-specific probe. The quantity of DNA can then be calculated through comparison of the signal to standards of known DNA quantity.

In order to achieve the best result with AluQuant™ we decided to use the Fluoroscan Ascent FL luminometer for the measurement of the light output. This instrument offers us the possibility to measure all samples under unchanged conditions due to the use of the built-up incubator, shaker and dispenser.

We followed the AluQuant™ manufacturer protocol with the exception of Luciferase/Luciferin reagent transfer to the 96 luminometer plate. The reactions were transferred to the luminometer plate first and the dispenser automatically injected the Luciferase/Luciferin reagent. The use of the dispenser, shaker and temperature control speeds up the whole process but keeps the reaction conditions constant for all samples.

The study on AluQuant™ performed in the Fluoroscan Ascent FL luminometer tested the performance, accuracy and limits of the quantitation system. We tested the AluQuant™ under the stress conditions that can occur during the forensic practice. Besides testing the effect of degraded DNA, inhibitors and DNA isolation technique used on the AluQuant™ performance, we also tested the cross-reactivity with non-human DNA and quantitated DNA bound to the FTA® paper matrix.

Conclusions:

The use of the Fluoroscan Ascent FL luminometer for the AluQuant™ Human DNA Quantitation System ensures that the reaction conditions are kept constant for all samples. The use of dispenser slightly increases the Luciferase/Luciferin reagent volume needed for the assay.

The AluQuant™ Human DNA Quantitation System was found as a simple and sensitive method for quantitating the concentration of human DNA in forensic samples and provides a versatile alternative to the forensic community.