

LightCycler Relative Quantification Software – a New Tool for Efficiency-adjusted Relative Quantification



The LightCycler system is the ideal tool for real-time, kinetic PCR quantification by allowing measurements to be made at each cycle during the log-linear phase of a PCR reaction

Quantitative real-time PCR can be subdivided into two basic classes of concepts:

→ Absolute quantification

This method is based on the use of a standard dilution series with predetermined known concentrations. The target concentration is expressed as an absolute value. An example for the usage of absolute quantification is determination of absolute numbers of infectious particles like viruses or bacteria in body fluids or other material. The LightCycler Software, distributed with the LightCycler Instrument, provides all the functions for a straightforward approach to an absolute quantification of a target, using an external standard curve.

→ Relative (comparative) quantification

The target concentration is expressed relative to the concentration of a reference gene in the same sample material. Therefore, relative quantification does not require standards with known concentrations. The method is recommended for determination of mRNA expression levels or gene doses values from tissue or cell cultures. Relative quantification of a target nucleic acid is also possible with the current LightCycler Software, but requires manual calculation.

The new LightCycler Relative Quantification Software is an additional software module that supplements the current software to expand the range of possible applications of the LightCycler system significantly. It represents an essential cornerstone of a novel approach to efficiency-adjusted relative quantification, developed at Roche Molecular Biochemicals: **The LightCycler Relative Quantification Software is designed to determine exact relative nucleic acid concentration, normalized to a calibrator sample.**

An outstanding feature of the new concept is that it acknowledges the fact that there may be differences in PCR efficiency, depending on nature of the particular target nucleic acid, reference (e.g., house-keeping gene) and other assay components. To address this fact, the software contains a newly developed efficiency-correction algorithm. The term “exact relative quantification” can only be applied when using this algorithm.

LightCycler Quantification Kits from Roche Molecular Biochemicals developed for use with the new software are provided with kit- and lot-specific coefficients for efficiency correction. The first kits following that new approach are the LightCycler-HER2/neu Quantification Kit and the LightCycler-CK20 Quantification Kit (see pages 14 and 18).

The concept also allows the adoption of the principles of the LightCycler Relative Quantification Software for designing user-specific assays: Relative standard dilutions using a typical nucleic acid are run and analyzed to create assay-specific coefficients for use in the software.

A detailed description of the underlying principles of the LightCycler Relative Quantification Software will be provided in the next issue of Biochemica.

<http://biochem.roche.com/lightcycler>



Product	Pack Size	Cat. No.
LightCycler Relative Quantification Software	1 software package	3 158 527

