



LightCycler-HER2/neu DNA Quantification Kit

Oncology research: It has never been easier and more accurate to quantify DNA encoding for human HER2/neu

The new LightCycler-HER2/neu DNA Quantification Kit allows the quantitative detection of DNA encoding for the human HER2/neu gene relative to a reference gene in a single reaction by dual color detection. Using the LightCycler Instrument as a closed-tube, rapid PCR amplification and real-time fluorescence detection system, the HER2/neu DNA levels can be specifically and quantitatively detected down to 10 copies in less than 70 minutes.

Background Information

The human epidermal growth factor receptor-2 (HER2, also referred to as HER2/neu or c-erbB2) gene is a proto-oncogene mapped on chromosome 17q21. It encodes a 185-kDa transmembrane cell surface glycoprotein, which is often called HER2/neu protein or receptor. HER2/neu is a member of the tyrosine kinase family with a high degree of homology with the human epidermal growth factor receptor (EGF-R). HER2/neu gene amplification and/or

overexpression is frequently observed in subsets of a wide range of human cancers (e.g., breast, ovarian, endometrial, cervical, lung, gastric, colon, mesenchymal, esophageal, bladder, kidney, head and neck squamous cell, pancreatic, salivary gland, and thyroid cancer).

Product Description

The LightCycler-HER2/neu DNA Quantification Kit is specifically adapted for PCR in glass capillaries using the

Table 1: The new LightCycler-HER2/neu DNA Quantification Kit

Assay characteristics

Analytical sensitivity

- Detects 10 copies of DNA encoding for human HER2/neu (in an exemplary system, using HER2/neu plasmid DNA as a reference).

Measuring range

- The linear measuring range of the assay is 10^2 - 10^6 copies of DNA encoding for human HER2/neu (in an exemplary system, using HER2/neu plasmid DNA).

Features and benefits

Quantitative results

- The kit allows the quantitative determination of DNA encoding for human HER2/neu relative to a reference gene and normalized to the amount of the Calibrator DNA.
Benefit: Correlation of HER2/neu DNA levels to other parameters.

Easy handling

- The kit contains all essential compounds as ready-to-use mixes.
- All reagents are function tested and provided in convenient ready-to-use solutions, enabling straightforward use of the kit without any further optimization.
Benefit: No expert knowledge required.

Fast performance / reduced handling

- Amplification and quantification are performed in a combined procedure on the LightCycler Instrument.
- PCR conditions are optimized to allow the simultaneous analysis of the HER2/neu and the reference gene within the same capillary.
Benefit: Time saving / reduced labour costs, i.e. results are obtained within 70 minutes.

Reliable performance / standardized kit concept

- Primers and Hybridization Probes are sequence-specific.
- FastStart Taq DNA Polymerase is used as 'Hot Start' component to achieve maximum specificity and sensitivity.
- The kit includes Calibrator DNA as a positive control template.
Benefit: Results are unambiguous and comparable between runs and individual samples.

LightCycler Instrument. A 112-bp fragment of HER2/neu gene and a 133-bp fragment of the reference gene from human genomic DNA that serves both as control for DNA integrity and as a reference for relative quantification are amplified by polymerase chain reaction (PCR) with specific primers. The amplicons are simultaneously detected in a single capillary by using two specific pairs of Hybridization Probes.

This simultaneous quantification is achieved because the HER2/neu-specific oligonucleotide is labeled at the 5'-end with LightCycler-Red 705, and the reference gene specific oligonucleotide is labeled at the 5'-end with LightCycler-Red 640. The use of a previously stored color compensation file is a prerequisite in this dual color experiment. To determine the HER2/neu DNA amplification level, the new LightCycler Relative Quantification Software is applied.

No post-PCR processing is needed and the risk of contamination is minimized, as quantification and detection are performed simultaneously in the same sealed capillary without any further handling steps. DNA isolated from cell cultures, scientific biopsy material, and other biological

samples (e.g., frozen or formalin-fixed paraffin-embedded tissue) can be used as sample material. For assay characteristics and features and benefits see Table 1.

For an extensive performance description of the LightCycler-HER2/neu DNA Quantification Kit see article "Realtime Quantification of HER2/neu Gene Amplification by LightCycler Polymerase Chain Reaction (PCR) – a New Research Tool" on this page of this Biochemica issue.

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



Product	Pack Size	Cat. No.
LightCycler-HER2/neu DNA Quantification Kit	1 kit (32 reactions)	3 113 922
LightCycler-Color Compensation Set	1 set (5 reactions)	2 158 850
LightCycler Relative Quantification Software	1 software package	3 158 527

Real-time Quantification of HER2/neu Gene Amplification by LightCycler Polymerase Chain Reaction (PCR) – a New Research Tool

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The tyrosine growth factor receptor HER2/neu is frequently overexpressed in breast cancer and other solid tumors, mostly due to gene amplification. A novel PCR method, the LightCycler-HER2/neu DNA Quantification Kit from Roche Molecular Biochemicals, has been evaluated to quantify HER2/neu gene copies in several breast cancer research samples. Unlike other PCR approaches [1-3] this assay uses a reference gene, which is co-localized to HER2/neu on chromosome 17. Consequently, it also corrects for the possible occurrence of polysomy of chromosome 17.

Introduction

The proto-oncogene HER2/neu is located on chromosome 17 (q21) and encodes a 185-kDa transmembrane glycoprotein with tyrosine kinase activity that is a member of the epidermal growth factor receptor family

[4-6]. Gene amplification and overexpression of HER2/neu is frequently observed in various human cancers (e.g., breast, stomach, ovarian, bladder, lung cancer). In breast cancer, for example, 10-34% of all affected individuals show this gene amplification/overexpression [7].