

cation in human tumor tissue. Samples negative and positive for HER2/neu overamplification can easily be discriminated. The results achieved are unambiguous and due to the kit set up comparable between runs and individual samples. The kit offers a fast detection method

for HER2/neu DNA overamplification as 30 samples can be tested per LightCycler PCR run in less than one hour. Further intensive research is required to determine the possible clinical usefulness of the real-time quantification of the HER2/neu gene amplification in tumor tissues.



Product	Pack Size	Cat. No.
LightCycler Instrument	1 instrument	2 011 468
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
High Pure PCR Template Preparation Kit	1 kit (100 purifications)	1 796 828

References

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3. Sestini, R. et al. (1995), *Clin. Chem.* 41(6): 826-832.
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<http://biochem.roche.com/lightcycler>



LightCycler-CK20 Quantification Kit

The most sensitive way to quantify RNA encoding for human cytokeratin 20

The new LightCycler-CK20 Quantification Kit from Roche Molecular Biochemicals expands the parameter-specific quantification reagents in the field of oncology research. This dedicated kit enables the quantitative detection of one CK20 positive cell in 1×10^6 peripheral blood cells.

Background Information

It is believed that many tumor recurrences, despite metastasis free intervals, occur due to the presence of micrometastases that are currently undetectable by conventional methods. Due to their increased sensitivity, PCR-based methods are being investigated as a possible tool for micrometastases detection. For these purposes, many markers are being evaluated for their suitability, with the prime candidates being those with restricted expression. Expression of these genes in extraneous environments is considered a potential indicator of tumor cell dissemination. The human cytokeratin 20 (CK20) belongs to the epithelial subgroup of the intermediate filament protein family that is involved in cytoskeletal structure. Like all other cytokeratins, its expression varies according to tissue type. Due to its restricted expression, CK20 has been studied as a candidate marker of micrometastases, primarily for colorectal and urothelial

cancer. CK20 detection has been correlated with increased recurrence risk and tumor stage.

Product Description

The LightCycler-CK20 Quantification Kit is specifically adapted for PCR in glass capillaries using the LightCycler Instrument. Detection of cytokeratin 20 (CK20) RNA is conducted by a two-step procedure. In the first step, cDNA is reverse-transcribed from RNA using AMV reverse transcriptase and random hexamer priming. In the second step, a 124-bp fragment of CK20-encoding mRNA is amplified from the cDNA by polymerase chain reaction (PCR) using specific primers. The amplicon is detected by fluorescence using a specific pair of Hybridization Probes. Using the same cDNA preparation but in a separate PCR reaction, mRNA encoding for porphobilinogen deaminase (PBGD) is processed as a reference gene. The reaction product serves both as a

Table 1: The new LightCycler-CK20 Quantification Kit

Assay characteristics
Analytical sensitivity → Detects ≤ 10 copies of CK20 nucleic acid sequences (in an exemplary system, using CK20 plasmid DNA as a reference).
Measuring range → The linear measuring range of the assay is 10 ² - 10 ⁶ CK20 copies (in an exemplary system, using CK20 plasmid DNA).
Features and benefits
Quantitative results → Quantitative determination of human CK20 mRNA levels relative to a reference gene and is normalized to the amount of the Calibrator RNA. Benefit: Correlation of CK20 mRNA levels to other parameters.
Easy handling → Ready-to-use mixes. → Reagents are function tested and provided in convenient ready-to-use solutions, enabling straightforward use of the kit without further optimization. Benefit: No expert knowledge required.
Fast performance / reduced handling → Amplification and quantification are performed in a combined procedure on the LightCycler Instrument. Benefit: Time saving / reduced labor costs, i.e. results are obtained within 120 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 RNA as a positive control template. Benefit: Results are unambiguous and comparable between runs and individual samples.

control for RNA integrity and as a reference for relative quantification. To determine CK20 mRNA quantities, the new LightCycler Relative Quantification Software is applied. For an extensive performance description of the LightCycler-CK20 Quantification Kit see article "Relative Quantification of Cytokeratin 20 on the LightCycler Instrument" on this page.

Product	Pack size	Cat. No.
LightCycler-CK20 Quantification Kit	1 kit (96 reactions)	3 118 835
LightCycler Relative Quantification Software	1 software package	3 158 527



Relative Quantification of Cytokeratin 20 on the LightCycler Instrument



An innovation providing new insights into the detection of micrometastases in research samples

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Introduction

Residual tumour cells, currently undetectable by conventional methods, may be responsible for cancer recurrences after apparent tumour clearance. The possibility of detecting these so-called "micrometastases" or "minimal residual disease" by RT-PCR amplification of tissue- or tumour-specific genes in extra-tumoural sam-

ples such as blood, bone marrow and lymph nodes has driven a growing amount of research in recent years [1]. A promising candidate marker of micrometastases is cytokeratin 20 (CK20). Expression studies found its expression restricted to gastrointestinal tissue, bladder transitional and Merkel cell carcinoma [2], therefore CK20 detection in extra-tumoural samples may be indicative of metastasis from these tumours. However, whilst some