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Multianalyte assay for prostate cancer-related gene quantification by hybridization on fluorescent microspheres

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Prostate cancer (PCa) is the second most common cancer in men and the fifth most common cancer overall. This fact necessitates early diagnosis, prognosis and monitoring of patients with prostate cancer. Consequently, there is a pressing demand for new sensitive and specific biomarkers. In this work, we developed a multiplex quantitative competitive polymerase chain reaction (QC-PCR) assay for simultaneous determination of 7 mRNA transcripts of PCa-related genes, including 5 genes from the kallikrein family (KLK3, KLK4, KLK5, KLK11 and KLK15), the prostate-specific membrane antigen (PSMA) and prostate cancer 3 (PCA3). In addition, the HPRT1 mRNA was quantified as a housekeeping gene. Isolated RNA from cell lines and clinical samples was first reverse transcribed followed by the addition of 8 synthetic DNA internal standards (DNA competitors) at a level of 5000 copies of each. DNA competitors had the same size with their respective targets, differing only in a 24-bp segment located in the middle of their sequence, thus allowing subsequent discrimination by hybridization. The addition of competitors allowed compensation for any variation of PCR efficiency. After amplification by multiplex PCR, the products were thermally denatured and hybridized with specific oligonucleotide probes immobilized on the surface of spectrally encoded fluorescent microspheres. All 16 DNA amplification products were biotinylated at the 5' end through biotin-modified primers. The hybrids were detected by using a streptavidin-phycoerythrin conjugate via streptavidin-biotin interaction. Finally, the microspheres were analyzed by flow-cytometry employing two laser beams. The first laser beam was used for the classification of microspheres into groups, thereby identifying the corresponding gene, while the second laser beam was used for the excitation of phycoerythrin, whose fluorescence was directly related to target concentration. We performed calibration graphs for the 16 PCR products demonstrating analytical ranges from 10 to 1000 pM. Extensive cross-hybridization assays were carried out, in which each target was hybridized separately with a pool of all 16 probes. These experiments proved the specificity of the assay.

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