Quantitative RT-PCR analysis with genomewide, validated primer sets

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Introduction

QuantiTect Primer Assays

QuantiTect® Primer Assays are bioinformatically validated, genomewide primer sets for use in real-time RT-PCR with SYBR® Green detection. The assays provide high specificity and sensitivity in gene expression analysis, and are ideal for applications such as validation of RNAi or microarray data. Assays are available for many species, including human, mouse, and rat.

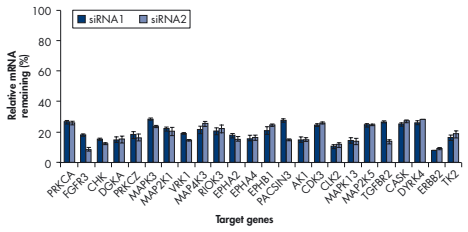
GeneGlobe™ Web portal

QuantiTect Primer Assays are easily ordered online at the GeneGlobe Web portal (www.qiagen.com/GeneGlobe). This comprehensive Web portal also offers a wide range of other gene-specific products, such as genomewide siRNAs, validated siRNAs, and protein assays.

QuantiFast™ and QuantiTect Kits

Highly specific and sensitive results are guaranteed when QuantiTect Primer Assays are used in combination with optimized master mixes from QIAGEN. These are QuantiFast SYBR Green Kits (for fast cycling) and QuantiTect SYBR Green Kits (for standard cycling). Both are compatible with any real-time cycler.

Reliable knockdown determination with QuantiTect Primer Assays



QuantiTect Primer Assays were used in SYBR Green based real-time RT-PCR to assess target gene knockdown in MCF-7 cells that were transfected 48 hours earlier with HP Validated siRNAs (QIAGEN). mRNA levels were normalized and calculated relative to levels in untransfected cells (set at 100%).

Intuitive online ordering of validated assays

QuantiTect Primer Assays for SYBR Green based real-time RT-PCR are carefully designed and rigorously validated by QIAGEN. Assays for genes of interest are easy to find and order at www.qiagen.com/GeneGlobe .

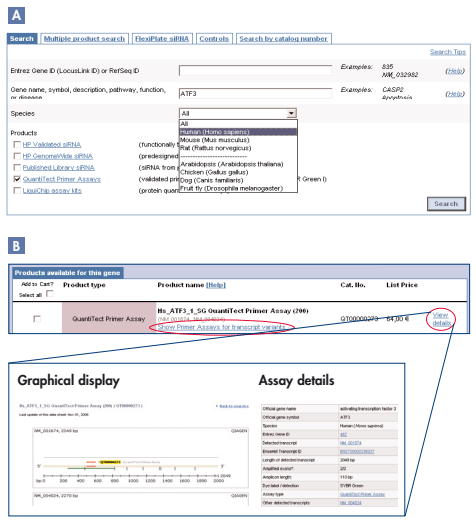
Criteria for successful validation:

- Single peak in melting curve analysis
- No primer–dimers in NTC (no template control)
- No amplification of genomic DNA (if applicable)
- PCR efficiency close to 100%
- Detection of 100 copies of target
- Similar performance in two-step and one-step RT-PCR
- Compatibility with all real-time cyclers

Design and validation process:

- Sequence retrieval from curated databases
- Exclusion of SNPs from assay design
- Design of RNA-specific assays with at least one primer overlapping a splice site
- In silico quality control of designed primer combination
- Testing of thousands of assays in wet lab experiments

Easy and intuitive ordering at the GeneGlobe Web portal

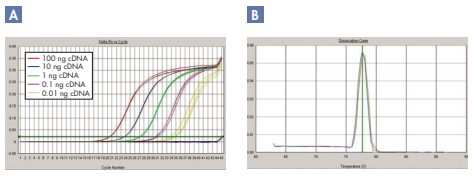


A Enter your gene name and select the species. Alternatively, upload a list with genes of interest. GeneGlobe searches for the matching assay. B Click "View details" to find out more about the assay. If available, click "Show Primer Assays for transcript variants" to view assays for transcript variants.

High sensitivity and specificity

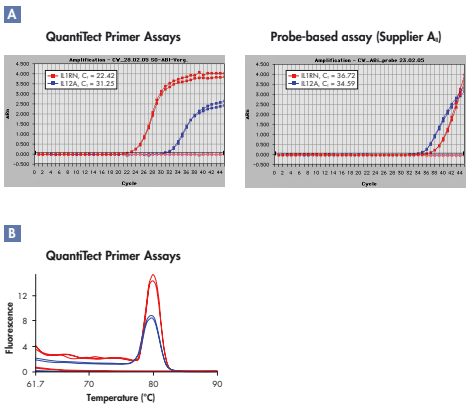
QuantiTect Primer Assays, when used in combination with QuantiFast/QuantiTect SYBR Green Kits, provide high PCR efficiency over a wide linear range. C_T values are lower than or comparable with those achieved with probe-based assays.

Reproducible C_T values over a wide linear range



A Different amounts of leukocyte cDNA were analyzed in triplicate using the QuantiTect Primer Assay for IL1RN and the QuantiTect SYBR Green PCR Kit. No template control (NTC) and genomic DNA control reactions were also performed (shown by the flat plots). B Melting curve analysis, demonstrating high PCR specificity.

Sensitivity comparable to probe-based detection

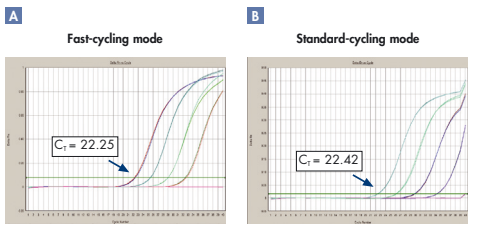


A IL1RN and IL12A in human leukocyte cDNA (10 ng) were quantified in duplicate using QuantiTect Primer Assays and the QuantiTect SYBR Green PCR Kit, or predesigned probe-based assays and a PCR kit from Supplier A. No template control (NTC) reactions were also performed (shown by the flat plots). B Melting curve analysis for the QuantiTect Primer Assays, demonstrating high PCR specificity.

Flexible use of primer assays

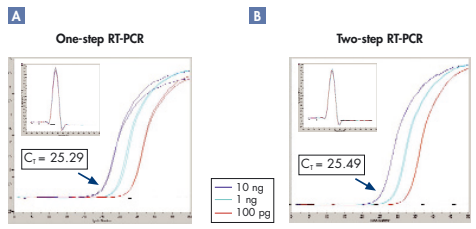
QuantiTect Primer Assays perform equally well under both standard-cycling and fast-cycling conditions. The assays also provide comparable performance in two-step and one-step RT-PCR procedures.

Comparable results in fast- and standard-cycling mode



Expression of MYC (a proto-oncogene) in human leukocytes was analyzed by real-time, two-step RT-PCR using the corresponding QuantiTect Primer Assay. Duplicate reactions were run using 10-fold cDNA dilutions (10 ng to 10 pg) on the Applied Biosystems® 7500 Fast System. A Fast-cycling mode with the QuantiFast SYBR Green PCR Kit gave similar C_T values to B standard-cycling mode with the QuantiTect SYBR Green PCR Kit.

Comparable results in one-step and two-step RT-PCR



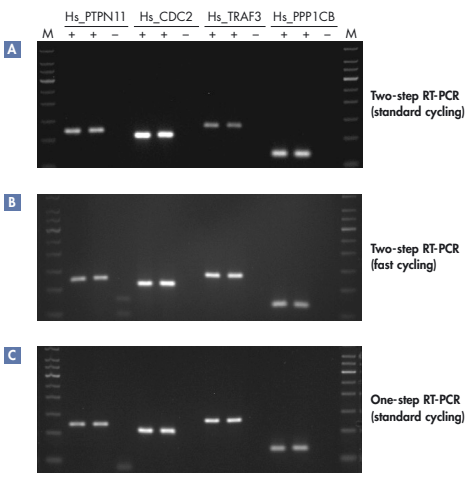
Expression of MAPK14 (a kinase) in HeLa cells was analyzed on the LightCycler® 1.5 using the corresponding QuantiTect Primer Assay. The template was A total RNA (10 ng to 100 pg) or B the equivalent amounts of cDNA.

End-point RT-PCR analysis

QuantiTect Primer Assays are intended for use in SYBR Green based real-time RT-PCR, and are also compatible with end-point RT-PCR. The RT-PCR products can be analyzed in applications such as gel electrophoresis and capillary electrophoresis, and can also be used to prepare PCR-generated detection probes for northern blot analysis.

For end-point RT-PCR analysis with QuantiTect Primer Assays, either one-step RT-PCR or two-step RT-PCR can be carried out. The latter procedure is possible both in standard-cycling mode as well as in fast-cycling mode on thermal cyclers with the appropriate QIAGEN PCR kit.

Highly specific end-point PCR analysis



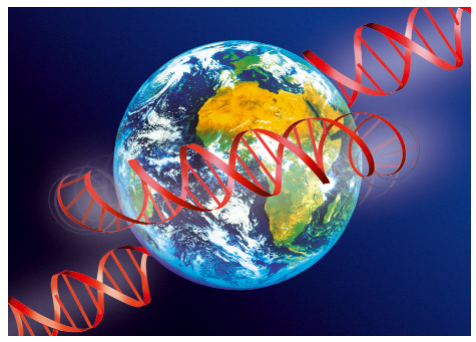
End-point RT-PCR analysis of the indicated genes was performed using the corresponding QuantiTect Primer Assay and 10 ng RNA or cDNA from HeLa cells. A Two-step RT-PCR with the Omniscript® RT Kit and HotStarTaq® Master Mix Kit. B Two-step RT-PCR with the Omniscript RT Kit and QIAGEN® Fast Cycling PCR Kit. C One-step RT-PCR with the QIAGEN OneStep RT-PCR Kit. **M**: marker; +: template added; –: no template control. **PTPN11**: a tyrosine phosphatase; **CDC2**: a cell-cycle protein; **TRAF3**: a receptor-associated protein; **PPP1CB**: a phosphatase.

Summary

QuantiTect Primer Assays provide:

- Significant time savings by eliminating the need for assay development
- PCR efficiencies of ~100%, allowing accurate relative quantification data
- High sensitivity and specificity, and accurate quantification over a wide linear range
- Cost savings through detection with SYBR Green instead of probes
- Reliable results in end-point RT-PCR analysis

GeneGlobe Web portal



Visit www.qiagen.com/GeneGlobe to order QuantiTect Primer Assays and other gene-specific products

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