

Amplification Products for PCR and RT-PCR

Quantitative Competitive PCR (QC-PCR)

Technical tip

RT-PCR and quantitation

The use of RT-PCR to examine levels of gene transcripts has become very popular because PCR is very sensitive and rapid. However, quantification of mRNA can be difficult due to the exponential nature of PCR, where small variations in amplification efficiency lead to dramatic changes in product yields. In addition, amount of product generated reaches a plateau during later stages of PCR. These characteristics can obscure differences in levels of the target mRNA during amplification.

QC-PCR PROTOCOL OUTLINE

Prepare Target Sequences

Isolate RNA

Synthesize 1st-strand cDNA

Conduct Competitive PCR

Make ten-fold serial dilutions of PCR Competitor

Add portion of each PCR Competitor dilution to 1st-strand cDNA samples

Perform PCR & electrophorese products on an agarose gel

Analyse above data. Make proper two-fold serial dilution of PCR Competitor with selected 10-fold dilution

Add dilution to cDNA samples, perform PCR, and electrophorese on an agarose gel

Outline for using a PCR competitor in competitive PCR to quantify mRNA levels. The target sequence is prepared by reverse transcription of RNA. To quantitate your target sequence, decreasing amounts of PCR competitor are added to PCR reactions containing a constant amount of target cDNA. Following PCR, the products derived from the competitor and target are resolved and amounts compared on an agarose gel.

Each kit contains sufficient for 100 tests :

- 2X QC-PCR Buffers
- QC-PCR Competitor
- QC-PCR Control
- 10X QC-PCR Primers
- ddH₂O (DNase free)
- Dilution solution (10X)
- Instruction manual

Quantitative Competitive PCR (QC-PCR)

PCR competitor are a series of DNA fragments for use as competitive internal controls in PCR. PCR competitor can be used to accurately measure small changes in RNA levels. To perform competitive PCR, known quantities of PCR competitor are added to PCR reaction containing test samples. A comparison of PCR products can be used to determine the quantity of target gene products. Quantitative competitive RT-PCR kits (QCRT-PCR) combine the core MPCRT technology with PCR competitors. This ready-to-use kit contains all reagents except Taq Pol. to perform 100x 50 ul QCRT-PCR.

Cat.# Description

Human

T66000	Human basic FGF, QCRT-PCR kit
T65880	Human bcl-2 gene, QCRT-PCR kit
T65890	Human bcl-xL gene, QCRT-PCR kit
T65900	Human bcl-xS gene, QCRT-PCR kit
T65910	Human BiP Protein (GRP78), QCRT-PCR kit
T66060	Human caspase-3 gene, QCRT-PCR kit
T66070	Human caspase-8, QCRT-PCR kit
T66080	Human caspase-9, QCRT-PCR kit
T65920	Human CCR-5 gene, QCRT-PCR kit
T65930	Human CD154 Gene (CD 40 Ligand), QCRT-PCR kit
T65940	Human CD40 Gene, QCRT-PCR kit
T65960	Human CXCR-4 gene, QCRT-PCR kit
T65980	Human Fas gene, QCRT-PCR kit
T65990	Human FasL Gene, QCRT-PCR kit
FL1440	Human GAPDH, QCRT-PCR kit
T66020	Human H18S Gene, QCRT-PCR kit
T66090	Human IFN-r gene, QCRT-PCR kit
T66110	Human IL-1a gene, QCRT-PCR kit
T66120	Human IL-1b gene, QCRT-PCR kit
T66130	Human IL-2 gene, QCRT-PCR kit
T66150	Human IL-4 gene, QCRT-PCR kit
S08950	Human IL-10 gene, QCRT-PCR kit
T66100	Human IL-12 gene, QCRT-PCR kit
S23170	Human iNOS gene, QCRT-PCR kit
T66170	Human LIF Human LIF, QCRT-PCR kit
AU2530	Human Low Density Lipoprotein-related Protein 1 (LRP1), QCRT-PCR kit
FL1430	Human Macrophage-Colony Stimulating Factor (M-CSF), QCRT-PCR kit
T66180	Human MCP-1, QCRT-PCR kit
FL1550	Human NF kappa-b Factor, p65, QCRT-PCR kit
FL1560	Human NF-AT, QCRT-PCR kit
T66210	Human TGF-b gene, QCRT-PCR kit
S08980	Human TNF-a gene, QCRT-PCR kit

Mouse

FL1500	Mouse Fas, QCRT-PCR kit
T66140	Mouse IL-2 gene, QCRT-PCR kit
S08960	Mouse IL-10 gene, QCRT-PCR kit
T66200	Mouse iNOS, QCRT-PCR kit
FL1450	Mouse MCP-1, QCRT-PCR kit
T66220	Mouse TGF-b gene, QCRT-PCR kit
FL1510	Mouse TNF-alpha, QCRT-PCR kit

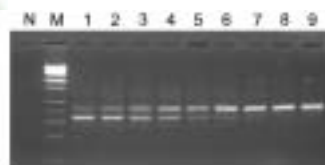
Rat

T66010	Rat Basic FGF Gene, QCRT-PCR kit
FL1460	Rat GAPDH, QCRT-PCR kit
FL1470	Rat GRO CINC-1 Gene, QCRT-PCR kit
T66160	Rat Insulin-1, QCRT-PCR kit
T66190	Rat rENaC, QCRT-PCR kit
FL1480	Rat Rod-type cyclic nucleotide-gated cation channel, QCRT-PCR kit
T66230	Rat TGF-b gene, QCRT-PCR kit
T66240	Rat TNF-a gene, QCRT-PCR kit

Viruses

T65950	Cytomegalovirus Matrix Protein (pp65), QCRT-PCR kit
T65970	Epstein-Barr Virus (EBV), BNF1, QCRT-PCR kit
T66030	HCV Unknown Coding Region, QCRT-PCR kit
T66040	HHV6 Alkaline Exonuclease (ORF16R), QCRT-PCR kit
T66050	HIV Gag, QCRT-PCR kit
FL1420	HBV Precore-Core Region, QCRT-PCR kit

Lane 1 : PCR with 106 copies of IL-12 gene plus 107 copies of Competitor.
 Lane 2 : PCR with 106 copies of IL-12 gene plus 5 x 106 copies of Competitor.
 Lane 3 : PCR with 106 copies of IL-12 gene plus 2.5 x 106 copies of Competitor.
 Lane 4 : PCR with 106 copies of IL-12 gene plus 1.3 x 106 copies of Competitor.
 Lane 5 : PCR with 106 copies of IL-12 gene plus 6.5 x 105 copies of Competitor.
 Lane 6 : PCR with 106 copies of IL-12 gene plus 3.3 x 105 copies of Competitor.
 Lane 7 : PCR with 106 copies of IL-12 gene plus 1.7 x 105 copies of Competitor.
 Lane 8 : PCR with 106 copies of IL-12 gene plus 8.5 x 104 copies of Competitor.
 Lane 9 : PCR with 106 copies of IL-12 gene plus 4.3 x 104 copies of Competitor.
 Lane N : PCR without template (Negative)



Quantitative analysis of Human IL-12 gene by QC-PCR.