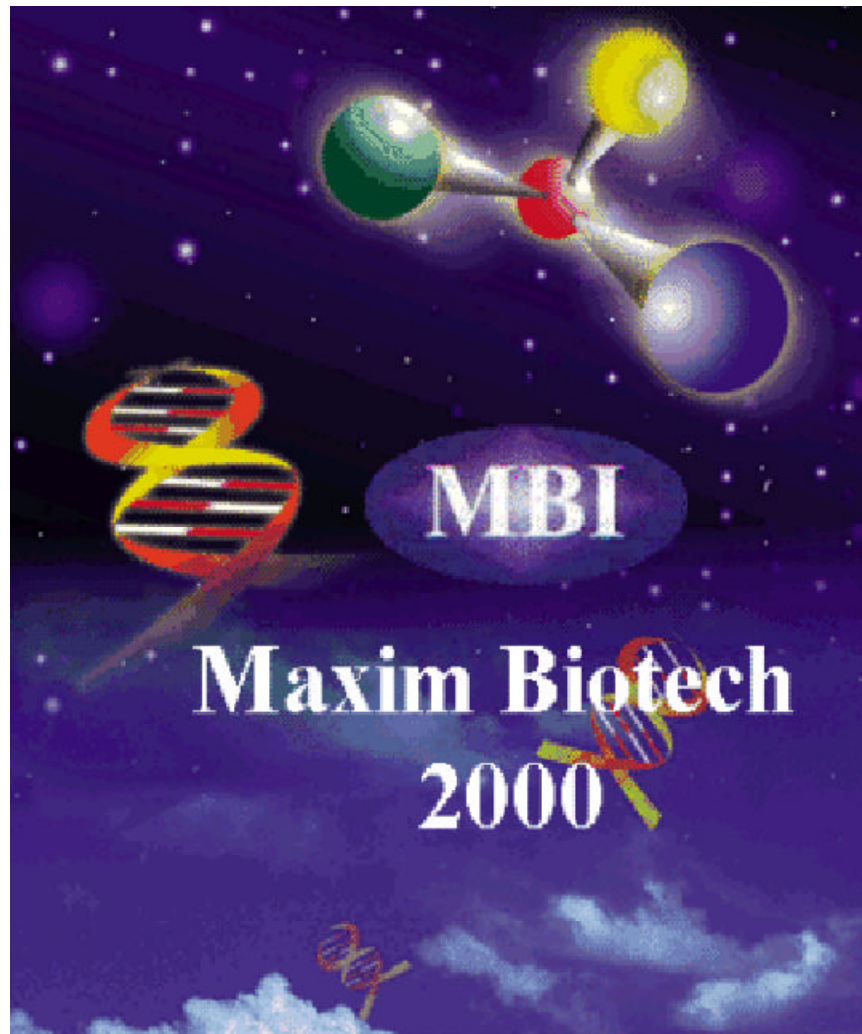


New Product Update

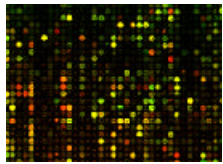


website: www.maximbio.com

Gene Expression Profiling

Analysis and comparison of when, where and to what degree genes are expressed, commonly known as expression profiling, provides the basis to further our understanding of the control and mode of action of individual gene products (1, 2). Nowadays DNA chip is the most widely used method for determining the abundance of thousands of genes in a total or poly(A) RNA sample (3). However, the application of DNA chip will be limited by its low sensitivity and high cost. DNA chip should be used as a discovery tool rather than as a daily assay. RT-PCR provides an alternate and accurate method to detect a specific gene expression (4). RT-PCR requires only a microgram or less of total RNA and only a few hours to perform. The recent advance in MPCR technology has allowed researchers to detect a group of genes at same time under the same condition (1, 5, 6). RT-MPCR fills the gap between DNA chip analysis and final conclusion, and is ideal for daily assay.

We recommend to use DNA chip first to identify genes related to a specific biological process, then use RT-MPCR to analyze these genes daily.



MPCR



Furthermore, variations in RNA isolation, initial quantification errors or tube-to-tube variations in both RT and PCR steps can be offset by including one of the house-keeping genes in RT-MPCR. Differences in gene expression can be determined by normalizing its expression against the house-keeping gene expression. However, expression of a putative "stable" housekeeping gene (GAPDH) can be actually varied as much as that of the target gene (7, 8). It becomes necessary to first detect several house-keeping genes expression under a specific system and chose the one without significant change in expression as a control in RT-MPCR. We have used our human house-keeping genes RT-MPCR kit (Cat # HKG-M052: 18S, L-32, Phospholipase A2, Transferrin Receptor and GAPDH genes) in several biology systems, and found that 18S expression was more "stable" during these biological process than other

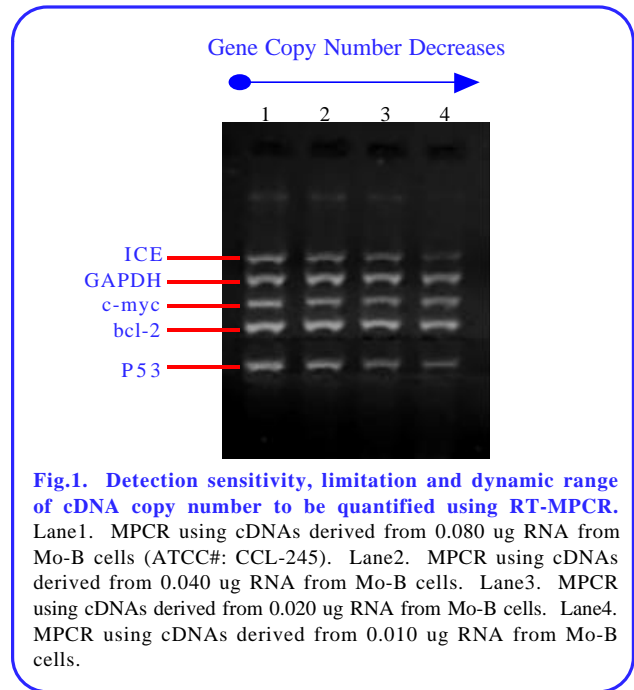
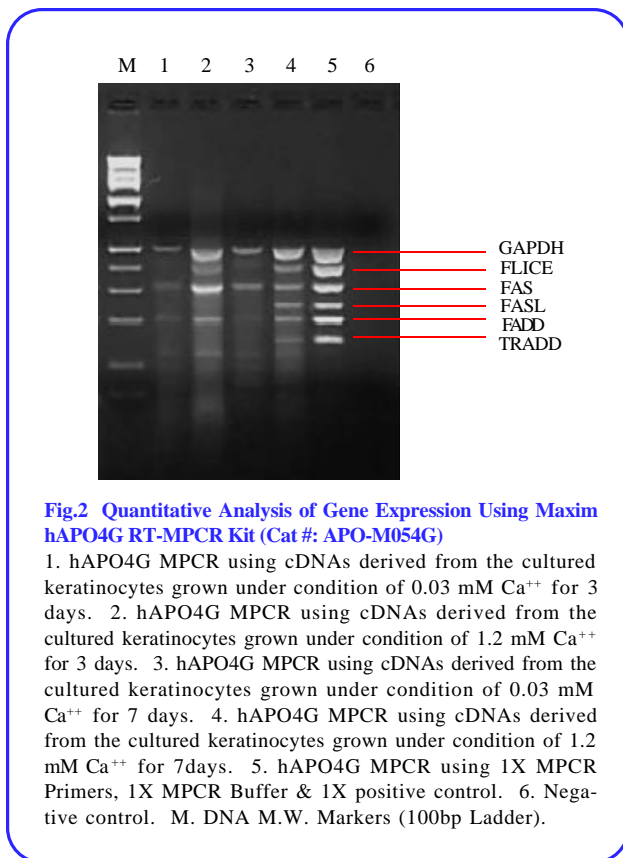


Fig.1. Detection sensitivity, limitation and dynamic range of cDNA copy number to be quantified using RT-MPCR. Lane1. MPCR using cDNAs derived from 0.080 ug RNA from Mo-B cells (ATCC#: CCL-245). Lane2. MPCR using cDNAs derived from 0.040 ug RNA from Mo-B cells. Lane3. MPCR using cDNAs derived from 0.020 ug RNA from Mo-B cells. Lane4. MPCR using cDNAs derived from 0.010 ug RNA from Mo-B cells.

genes. Therefore, we have incorporated 18S gene into some of our new RT-MPCR kits.

Maxim was the first to offer you the convenience and reliability of RT-MPCR Kits. This economical approach to expression profiling uses MMLV Reverse Transcriptase for reverse transcription followed by Multiplex PCR amplification. **Relative expression of gene groups with expression changes as low as 2-fold can be determined using Maxim's RT-MPCR kit as illustrated in Fig. 1.**

To examine the ability of RT-MPCR to accurately measure relative change in the levels of a specific mRNA, we applied our RT-MPCR kit to study the expression change of apoptotic genes during human keratinocytes differentiation. It is well known that extracellular calcium concentrations (Cao) > 0.1 mM are required for the differentiation of normal human keratinocytes in culture. Increments in Cao result in acute and sustained increases in the intracellular calcium level (Cai), postulated to involve both a release of calcium from intracellular stores and a subsequent increase in calcium influx through nonspecific cation channels. The sustained rise in Cai appears to be necessary for keratinocyte differentiation. To imitate a defined induction in these mRNA by Ca⁺⁺, we performed RT-MPCR with



cDNAs from different stages of keratinocytes. Keratinocytes were grown in keratinocyte growth medium with 0.03 mM, or 1.2 mM Ca⁺⁺. Cells are harvested at day 3 and day 7, respectively. We then extracted total RNAs, synthesized cDNAs, and performed RT-MPCR. Four identical experiments, each starting with the reverse transcription, were performed to determine the reproducibility of the overall method.

The EtBr-staining pattern obtained from one of the four independent experiments is shown in Figure 2. In our experience, visual inspection of an EtBr-stained agarose gel is sensitive and precise enough to detect changes as low as two-fold. If greater discrimination is necessary, several methods are available. The simplest procedure is to add a radioactively labeled dNTP to the PCR reaction. After gel analysis, the band may be excised and counted in a scintillation counter. Alternatively the gel may be dried and an autoradiogram may be generated which can be scanned in a densitometer.

The expression of each gene can be normalized by either calculating its percentage against GAPDH band in same lane after scanning gel band intensity, or directly compar-

ing each other's intensity after adjusting GAPDH band intensity to be similar in same gel. It can be seen from Fig.2. that there are considerable changes in level of FasL gene, subtle changes in levels of Tradd, Flice and Fadd genes, and not much changes in level of Fas gene.

For absolute quantification of gene expression, we offer QCRT-PCR Kits and Real Time PCR Kits (9, 10). Please contact Maxim for further information

In conclusion, there is a wide choice of methods available to monitor gene expression. The choice of methods shall depend on the particular features of the experiment and project requirement. There is no technique which could be considered the best for any task.

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RT-MPCR Features

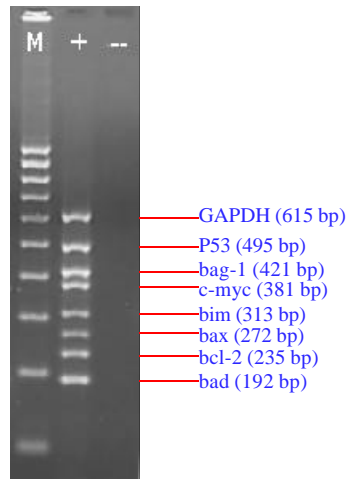
- ✓ **Multiple Gene Analysis**
- ✓ **Perform 1 Instead of 5-10 PCR**
- ✓ **Sensitive and Rapid**
- ✓ **Detect Subtle Differences In Gene Expression**
- ✓ **No Isotope**



Apoptosis Set-1 New

MPCR Kit Cat #: APO-M051G, APO-M011G

• P53 • Bag-1 • c-myc • bim • bax • bcl-2 • bad • GAPDH



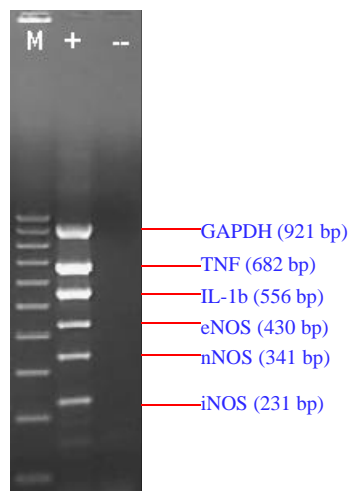
Maxim's APO-M051G RT-MPCR kits have been designed to detect the expression of human bad, bcl-2, bax, bim, c-myc, bag-1, P53 and GAPDH genes. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification. The 615 bp(GAPDH), 495 bp(P53), 421 bp(bag-1), 381 bp(c-myc), 313 bp(bim), 272 bp(bax), 235 bp(bcl-2) and 192 bp(bad) PCR products can be generated from human RNA. Therefore, the APO-M051G RT-MPCR kit provides a quick and simple method to analyze human bad, bcl-2, bax, bim, c-myc, bag-1, P53 and GAPDH genes expression during apoptosis & normalize their expression against GAPDH gene.



NO Metabolism

MPCR Kit Cat #: NOS-M050G, NOS-M010G

• nNOS • iNOS • eNOS • IL-1 β • TNF • GAPDH



NO is a signaling molecule that elicits numerous biochemical responses. NO exhibits contradictory effects in the regulation of apoptosis. Both pro- and anti-apoptotic effects have been demonstrated. The pro-apoptotic effects seem to be linked to pathophysiological conditions, where high amounts of NO are produced by the inducible nitric oxide synthase. In contrast, the continuous release of endothelial NO inhibits apoptosis and may contribute to the anti-atherosclerotic function of NO. Nitric oxide (NO) is produced by iNOS, eNOS and nNOS. IL-1 β is a potent inducer of iNOS. TNF- α might induce the production of NO and free radicals.

Maxim's NOS RT-MPCR kits have been designed to detect the expression of these genes related to NO metabolism. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification. Therefore, the NOS-M050G RT-MPCR kit provides a quick method to analyze human TNF- α , IL-1 β , eNOS, nNOS and iNOS gene expression, and normalize their expression against GAPDH.

Telomerase

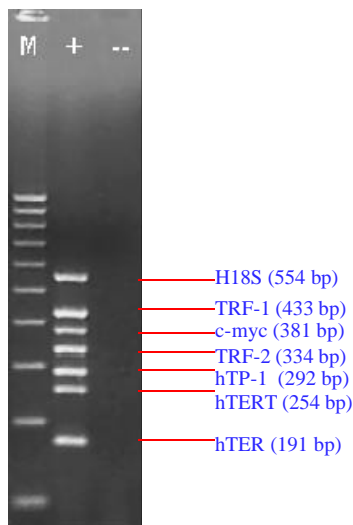
New Product



Telomerase Genes

MPCR Kit Cat #: TEL-M050S, TEL-M010S

- *hTER* • *hTERT* • *hTP-1* • *TRF-1* • *TRF-2* • *c-myc*
- *H18S*



Major advances have been made during last few years in understanding the link between telomerase expression and cell immortality. Telomerase is an unusual reverse transcriptase that contains an RNA molecule as well as various protein subunits. During synthesis of new telomeric DNA, the catalytic subunit utilizes a small templating domain in the RNA component to copy additional telomeric repeats onto the end of chromosome.

Human telomerase consists of an essential RNA subunit (hTER), the reverse transcriptase subunit (hTERT) and accessory proteins (hTP). The activity of telomerase is modulated by other proteins, such as TRF-1 and TRF-2. Several studies have shown that there is a good correlation between expression of hTERT mRNA and the presence of telomerase activity in extracts made from culture cells and normal and cancer tissues. How hTERT is upregulated is not yet understood but, in some cases, the elevated expression may be caused by an increase in the level of c-Myc.

Maxim's TEL-M050S MPCR kits have been designed to detect the expression of human hTER, hTERT, hTP1, TRF-1, TRF-2, c-Myc and H18S genes. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification in a single tube; The kit will yield 554 bp(H18S), 433 bp(TRF-1), 381 bp(c-Myc), 334 bp(TRF-2), 292 bp(hTP1), 254 bp(hTERT) and 191 bp (hTER) PCR products with RNA from human cells or positive controls from kit. The gene expression of these genes can be analyzed and compared with H18S gene expression.

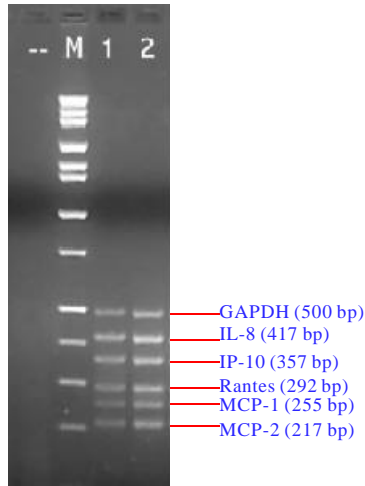
Chemokines

NEW

Chemokines Set-1

MPCR Kit Cat #: hCK-M050G, hCK-M010G

•IL-8•IP-10•Rantes•MCP-1•MCP-2•GAPDH



Chemokines & their receptors are important elements for the activation and selective attraction of various subsets of leukocytes, and play a critical role in controlling the movement of these cells during inflammation. Evidence gathered during past few years suggests an important role for chemokines in a variety of pathophysiological processes (e.g., chronic and acute inflammation, infectious diseases, modulation of angiogenesis, tumor growth, and hematopoietic progenitor cell proliferation). The most notable of these recent discoveries is that certain chemokine receptors function as co-receptors for HIV-1. Moreover, mutations in these receptors can result in host resistance to infection and also effect the progression of disease course.

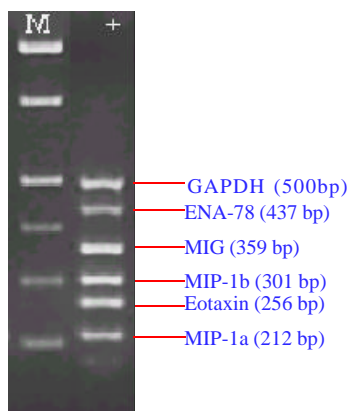
Maxim's hCK1G-MPCR kits have been designed to detect the expression of human IL-8, IP-10, Rantes, MCP-1, MCP-2 and GAPDH genes. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification in a single tube; The kit will yield 500 bp(GAPDH), 417 bp(IL-8), 357 bp(IP-10), 292 bp(Rantes), 255 bp(MCP-1) and 217 bp (MCP-2) PCR products with RNA from human cells or positive controls from kit. The gene expression of these genes can be analyzed and compared with GAPDH gene expression.

NEW

Chemokines Set-2

MPCR Kit Cat #: hCK-M052G, hCK-M012G

•ENA-78 •MIG •MIP-1b •Eotaxin •MIP-1a
•GAPDH



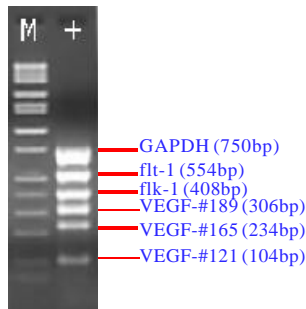
Maxim's hCK2G-MPCR kits have been designed to detect the expression of human MIP-1a, MIP-1b, Eotaxin, MIG, ENA-78, and GAPDH genes. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification in a single tube; The kit will yield 500 bp(GAPDH), 437 bp(ENA-78), 359 bp(MIG), 301 bp(MIP-1b), 256 bp(Eotaxin) and 212 bp (MIP-1a) PCR products with RNA from human cells or positive controls from kit. The gene expression of these genes can be analyzed and compared with GAPDH gene expression.



VEGF Set-1

MPCR Kit Cat #: VEGF-M050G, VEGF-M010G

- *FLT-1* • *FLK-1* • *VEGF-#189, #165, #121*
- *GAPDH*



Angiogenesis process is tightly regulated and depends on a dynamic balance between stimulators and inhibitors. The vascular endothelial growth factor (VEGF) family of proteins, together with their receptors are among the most studied stimulators, and play a crucial role in embryonic development and angiogenesis. Three VEGF receptors have been identified as Flt-1, Flk-1 and Flt-4. The Tie (tyrosine kinase with immunoglobulin and epidermal growth factor homology domains) receptors, and its ligand angiopoietin-1 control blood vessel formation as well.

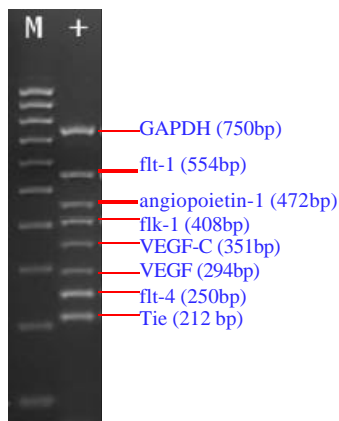
VEGF Set-1 RT-MPCR kit has been designed to detect the expression of human VEGF #165, #121, #189 and VEGF receptors flt-1 and flk-1 genes. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification. The 750 bp (GAPDH), 554 bp (flt-1), 408 bp (flk-1), 306 bp (VEGF #189), 234 bp (VEGF #165) and the 104 bp (VEGF #121) PCR products can be generated from human RNA or the positive control, which is included in this kit. Therefore, the expression of human VEGFs and its receptors genes can be monitored and normalized against the GAPDH expression.



VEGF Set-2

MPCR Kit Cat #: VEGF-M052G, VEGF-M012G

- *FLT-1* • *FLK-1* • *FLT-4* • *VEGF* • *VEGF-C*
- *TIE* • *Angiopoietin* • *GAPDH*



VEGF Set-2 RT-MPCR kit has been designed to detect the expression of human VEGF receptors flt-1 and flk-1, VEGF-C, VEGF (all sub-types), FLT-4, angiopoietin-1, TIE and GAPDH genes. The PCR primers have similar T_m and no obvious 3'-end overlap to enhance multiple amplification. The 750 bp (GAPDH), 554 bp (flt-1), 472 bp (angiopoietin-1), 408 bp (flk-1), 351 bp (VEGF-C), 294 bp (all sub-types of VEGF), 250 bp (FLT-4) and the 212 bp (TIE) PCR products can be generated from human RNA or the positive control, which is included in this kit. Therefore, the expression of human VEGF family and their receptors genes can be monitored and normalized against the GAPDH expression.



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PCR* Kits

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- ✓ Data sheet of optimal conditions supplied

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