



Human House Keeping Genes Multiplex PCR Control Kit

Catalog Number MG-0001 (For Research Use Only)

Introduction

Normalization with housekeeping genes (HKGs) such as GAPDH, 18SrRNA, SDHA, HPRT1, PP1A, B2M, HMBS, or RP43A, is required for the normalization of gene expression. This is because HKGs are relatively steady in the expression among different tissues. However, recent studies have showed that the use of a single HKG might not be sufficient to achieve the normalization of gene expression in some tissues, because its expression can be quite different. For example, there is a 15-fold difference of GAPDH mRNA between skeletal muscle and breast. In this case, it is required to choose a different HKG that is relatively steady in the expression in these two tissues for normalization. Therefore, simultaneous employment of different HKGs together will facilitate the quantitative analysis of gene expression. Signosis has developed a Human HKGs Multiplex PCR kit to monitor the gene expression of seven different HKGs, including GAPDH, 18srRNA, SDHA, HPRT1, B2M, HMBS, RPLI3A. The kit provides the optimized ready-to-use PCR mix containing polymerase and dNTP, and the primer mix with optimized concentration.

Principle

Multiple targets are amplified simultaneously with different primers in one PCR reaction. The resulted products with differential sizes are easily distinguished with regular agarose gel electrophoresis. The parameters of PCR including the primer concentration and the reaction buffer are optimized in order to provide the highest specificity and sensitivity of amplification of multiple targets in one reaction.

Materials provided

- Control cDNA mix
- Human PCR primer mix for GAPDH, 18srRNA, SDHA, HPRT1, B2M, HMBS, RPLI3A
- PCR buffer mix

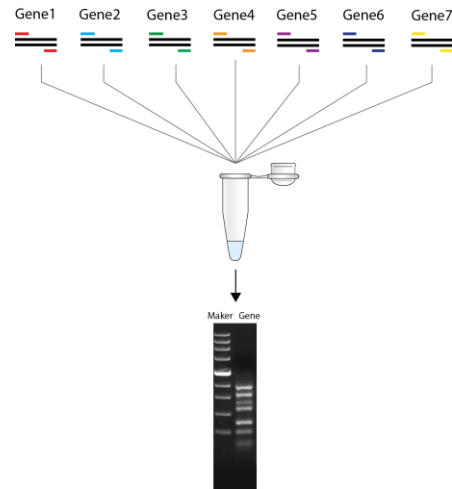


Diagram of Multiplex PCR Kit

Material may required but not provided

- Sample cDNA
- PCR machine

PCR amplification

- (1) Prepare PCR reactions
Mix the following component for one reaction:
18.8 ul PCR buffer mix
0.5 ul control cDNA mix or specific cDNA
0.5 ul PCR primer mix
0.2 ul PCR Polymerase
Note: make a master mix by multiplying the volume by the number of your reactions

- (2) Proceed PCR cycles:
Heat the reactions at 94 °C for 30 sec, and proceed
PCR for 35 cycles as follows:
94 °C 30 seconds
58 °C 30 seconds
72 °C 30 seconds
Note: PCR cycle can be adjusted according to a
specific primer designing.

(3) Run PCR products on 1.5% agarose gel
electrophoresis.

Data example

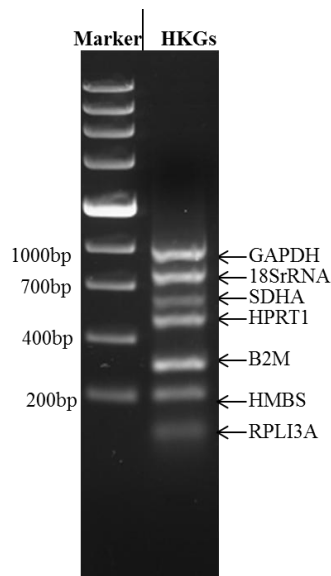


Figure: Human Housekeeping Multiplex PCR Control Kit, subjected to PCR for Multiplex Housekeeping genes with 35 cycles