53833 BioStab improved PCR Optimizer (II)

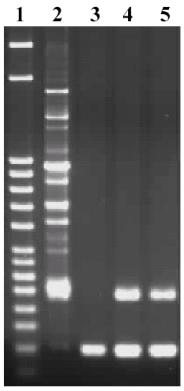
PCR is a generally robust technique extensively used in different applications in molecular biology and molecular diagnostics. The available enzymes and enzyme mixtures are very efficient in amplifying even very long PCR targets. However, some regions – those rich in GC pairs (for example, 5´-regions of many eukaryotic genes) – often prove to be refractory to amplification. The reason for that is their high melting temperature which leads to incomplete separation of DNA strands after the denaturation step and results in little or no yield of desired product and multiple nonspecific bands in agarose gels. Additives like formamide, DMSO and glycerol which affect DNA melting temperature have been used in PCR to solve this problem. However, the first two are toxic and inhibit DNA polymerase, while glycerol substantially increases viscosity of the reaction mix. Several companies provide non-toxic additives for PCR which are mainly based on betaine. We have developed an additive which outperforms betaine in amplification of more problematic targets (see Fig. 1 and 2) and is non-toxic as well.

Product Description:
The mode of action of BioStab PCR optimizer comprises lowering the melting temperature of GC pairs without affecting that of AT pairs.

E.g., human retinoblastoma gene contains a region at its 5´-end that is 75% GC and cannot be amplified without special additives to the PCR mixture (Fig 1, lane 2). Addition of the BioStab PCR optimizer to the PCR mixture in the final concentration of 1x allows efficient amplification of the desired 180 bp fragment (Fig 1, lane 3). Addition of either competitor´s product or 1M betaine produces additional non-specific band as well as the desired product (Fig 1, lanes 4 and 5). Targets with GC-content higher than 75% may require addition of double amount of BioStab PCR optimizer. An attempt to amplify a 149-bp region of human genomic DNA containing
78.5% of GC pairs and a CGG trinucleotide repeat in regular PCR buffer without additives leads to multiple non-specific products (Fig. 2, lane 2). The specific product free of any impurities was obtained when 2x concentrated
BioStab PCR optimizer was included in the PCR reaction (Fig.2, lane 3). Inclusion of either a competitor´s product or 1M betaine in the PCR mixture resulted in the amplification of non-specific products in addition to the
desired product (Fig 2, lanes 4 and 5). BioStab PCR optimizer allows the use of a wide range of annealing temperatures thereby eliminating the need for an optimization of amplification conditions (Fig 3).

Directions:
The BioStab PCR optimizer is provided as 5x concentrated solution in water. Use 1x or 2x final concentration in PCR reaction depending on the GC content of the target sequence. For targets with >75% GC content 2x solution is recommended.

Fig. 1: Amplification of a 180 bp fragment from the human retinoblastoma gene (75% GC).



lane 1: molecular weight marker – Perfect DNA™ 50 bp ladder, Novagen

lane 2: PCR without any additives to the PCR buffer

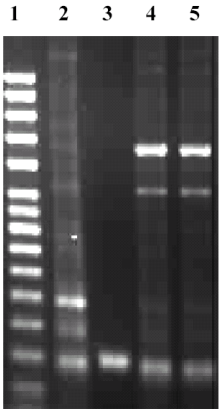
lane 3: PCR in the presence of 1xBioStab PCR optimizer

lane 4: PCR in the presence of competitor´s product in recommended concentration

lane 5: PCR in the presence of 1M betaine.

50 µl PCR mixtures contained 100 ng human genomic DNA, Qiagen PCR buffer (providing 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.5 units of Taq DNA polymerase (Qiagen) and 1 µM of each primer. PCR program was as follows: 95˚C, 3 min, 30 x (95˚C, 1 min; 59˚C, 1 min; 72˚C, 30 s), 72˚C, 10 min. 5µl of a PCR reaction was applied per lane of 2% agarose gel.

Fig. 2: Amplification of a 149 bp fragment from the human AF064849 locus (78.5% GC).



lane 1: molecular weight marker marker – Perfect DNA™ 50 bp ladder, Novagen

lane 2: PCR without any additives to the PCR buffer

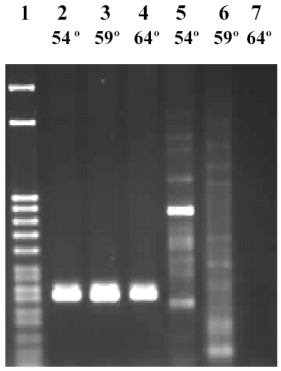
lane 3: PCR in the presence of 2xBioStab PCR optimizer

lane 4: PCR in the presence of competitor´s product in recommended concentration

lane 5: PCR in the presence of 1M betaine

50 µl PCR mixtures contained 100 ng human genomic DNA, Qiagen PCR buffer (providing 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.5 units of Taq DNA polymerase (Qiagen) and 1 µM of each primer. PCR program was as follows: 95˚C, 3 min, 32 x (95˚C, 1 min; 58˚C, 1 min; 72˚C, 30 s), 72˚C, 10 min. 5µl of a PCR reaction was applied per lane of 2% agarose gel.

Fig. 3: Amplification of a 375 bp fragment from the 5´region of mouse cAMP-dependent protein kinase Cß gene (74% GC) using various annealing temperatures.



lane 1: molecular weight marker marker – Perfect DNA™ 50 bp ladder, Novagen

lanes 2-4: PCR in the presence of 1xBioStab PCR optimizer

lanes 5-7: no additive to PCR mixture.

50µl PCR mixtures contained 100 ng mouse genomic DNA, Qiagen PCR buffer (providing 1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.5 units of Taq DNA polymerase (Qiagen) and 1 µM of each primer. PCR program was as follows: 95˚C, 3 min, 30 x (95˚C, 1 min; $T_{annealing}$, 1 min; 72˚C, 30 s), 72˚C, 10 min. 5µl of a PCR reaction was applied per lane of 1.5% agarose gel.

Precautions and Disclaimer:

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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operates as MilliporeSigma in the US and Canada.