MβP®
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EasyStart™ PCR Mix-in-a-Tube Makes PCR Reactions Easier than Ever Before.

ABSTRACT

EasyStart tubes, manufactured by Molecular Bio-Products, inc., were tested under various conditions for accuracy and stability of PCR reactions. EasyStart tubes were found, in every case, to produce results equal to, or better than those obtainable using standard protocols.

INTRODUCTION

EasyStart PCR Mix-in-a-Tube is a revolutionary new approach to performing PCR. This new technology enhances accuracy and yield while greatly reducing the time and cost required to set up reactions.

EasyStart tubes are pre-sterilized and available for 20, 50 and 100 µl reactions with pre-aliquoted PCR master mix hermetically sealed under a wax barrier. This unique design simplifies the setup process, reduces contamination risk and eliminates the need to store frozen PCR reagents.

OBJECTIVE

EasyStart's wax barrier protects PCR reagents and prevents degradation even at room temperatures. To ensure that accuracy was not sacrificed for convenience, shelf life was tested under various conditions.

To test stability and consistency, EasyStart 50 tubes were stored at room temperatures and then used to perform standard PCR using different DNA templates. The following experiments were conducted to illustrate the accurate and consistent results these convenient reaction tubes provide.

MATERIALS AND METHODS

EasyStart 50 tubes were stored on a bench top at 20-25° C for three months and then used to perform the following experiments.

Experiment #1
Primers specific for human mitochondrial D-loop were used with 0.02 µg total human DNA (Boehringer-Mannheim) and 1 unit of Taq polymerase (Life Technologies) in a standard PCR reaction of 94° C for 3 minutes, followed by 30 cycles of 56° C for 1 minute, 72° C for 1 minute, and 94° C for 30 seconds. The resulting 396 bp bands were visualized on a 1% agarose gel and run at 100 volts for 30 minutes.

Experiment #2
Additional EasyStart 50 tubes contained primers specific for human heat shock protein gene hsp 60 with 0.02 µg of human DNA. A standard PCR reaction was performed as described in experiment 1 and the resulting 1.7 kb bands were visualized on 1% agarose gel, run at 100 volts for 30 minutes.

RESULTS

In experiment #1, EasyStart produced superior results over standard protocols as shown by the brighter band intensity in lanes A-4, A-5 and A-6 (*figure 1*). These results confirm that EasyStart tubes can be stored at room temperature until ready to use without affecting the contents of the tube or the results of the reaction.

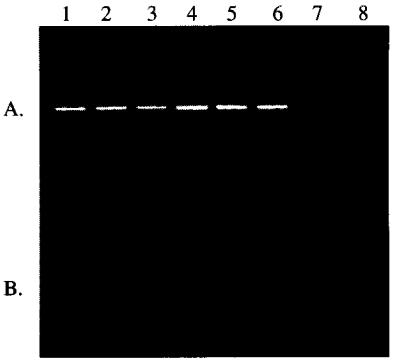
In experiment #2, EasyStart performed consistently but with greatly enhanced results over standard protocols. As shown in lanes B-4, B-5 and B-6, EasyStart tubes containing hard-to-amplify human heat shock protein produced enhanced specificity and yield (*figure 1*). When amplified on 1% agarose gel, EasyStart produced bands with greater intensity than did the fresh ingredients.

DISCUSSION

EasyStart tubes perform consistently and accurately after being stored for long periods at room temperature. This consistency is achieved through precise formulation of the lower layer reagent mix and careful assembly in a controlled environment. This means fewer variables and decreased contamination risk that will enable both low and high volume PCR labs to obtain precise and repeatable results.

Although routine, polymerase chain reactions can be complicated by time-consuming setup procedures, chemical breakdown of frozen components and errors caused through tedious protocols. The EasyStart advantage is obvious. EasyStart's one-step protocol and precise formulation result in increased specificity and yield and greater batch consistency than can be achieved through traditional methods.

Figure 1



A. Primers specific for a 396 bp fragment of human mitochondrial D-loop DNA.

B. Primers specific for a 1700 bp human heat shock protein hsp 60.

Lanes 1-3: PCR results using standard procedures.

Lanes 4-6: PCR results using EasyStart.

Lane 8: 1 kb ladder.