

**EasyStart™  
PCR Mix-in-a-Tube  
Compatible with Major  
Manufacturers' Taq DNA  
Polymerases**

**ABSTRACT**

EasyStart tubes were tested to evaluate their ability to work with most major manufacturers' Taq DNA Polymerases. To demonstrate this we amplified a region of human BRCA1 gene and compared the results from the EasyStart tubes to those received from using the manufacturers' prescribed buffer and reaction conditions. In each case, EasyStart performed as well or better than the manufacturers' included protocols.

**INTRODUCTION**

Enzyme manufacturers will typically guarantee an enzyme's performance only when it is used under their prescribed conditions. However, these conditions are rarely the only optimal environment for the enzyme. For example, with Taq DNA Polymerase, different PCR buffers will vary in salt concentration, pH, and MgCl<sub>2</sub> concentration.

EasyStart's pre-aliquoted, non-specific reagents allow for time savings and decreased contamination risk when performing PCR. While the buffer salt concentrations ([MgCl<sub>2</sub>] = 2mM) and pH of EasyStart are similar to those from most Taq manufacturers, we have chosen a hard-to-copy DNA fragment (BRCA1) to demonstrate that EasyStart tubes are compatible with different Taq DNA Polymerases regardless of the manufacturers' recommended conditions.

**OBJECTIVE**

We will perform a series of amplifications using different manufacturers' Taq DNA Polymerases (see Table A) to show that EasyStart will function as well or better than the manufacturers' suggested protocol. Each test will utilize the same primer sets and template DNA.

**MATERIALS AND METHODS**

In each experiment, three 50 μl EasyStart

reactions and three 50 μl standard PCR reactions were performed on the BRCA1 gene utilizing BR5 and BR3 primers specific for exon 11E2 (0.025 μg DNA)<sup>1,2</sup>. The standard PCR reactions utilized the buffer and protocol provided by the respective Taq DNA Polymerase manufacturers with a mineral oil overlay. The EasyStart reactions were performed according to the recommended protocol from Molecular Bio-Products, inc.

Thermal cycling consisted of one minute at 94° C followed by 30 cycles: 94° C, one minute; 56° C, one minute; 72° C, one minute.

The resulting PCR products were analyzed on a 1% agarose gel, run at 100V for 30 minutes.

**RESULTS**

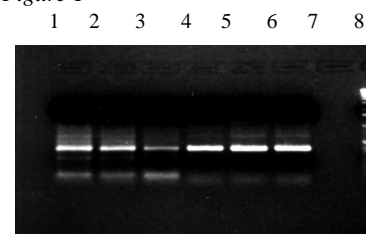
EasyStart proved to be compatible with the complete range of tested Taq DNA Polymerases. In fact, EasyStart returned superior results as compared to reactions performed with the buffers and protocols recommended by each enzyme manufacturer (see figures 1-6). Overall, the reactions performed with EasyStart did not show the primer dimerization apparent with conventional protocols and required considerably less time to prepare.

**DISCUSSION**

EasyStart's lower layer reagent mix is precisely formulated to provide consistent results and is optimum for nearly all reaction conditions. As demonstrated by the results of this test, the buffer, dNTPs and 2mM MgCl<sub>2</sub> are optimized to provide superior results with a wide range of enzymes.

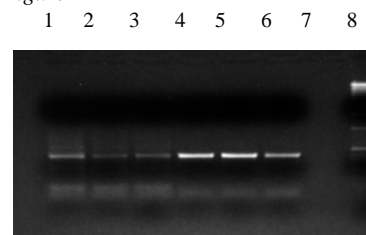
In addition, EasyStart's design facilitates a hot start reaction thereby improving the specificity and yield of each reaction. Because EasyStart allows the use of various Taq DNA Polymerases, there is little need to alter your PCR protocol to receive significantly improved results.

Figure 1



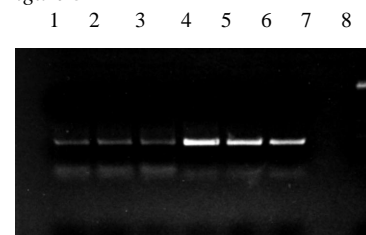
Taq DNA Polymerase from LifeTechnologies: Lanes 1-3, standard PCR; Lanes 4-6, EasyStart PCR; Lane 7, empty; Lane 8, 1kb ladder.

Figure 2



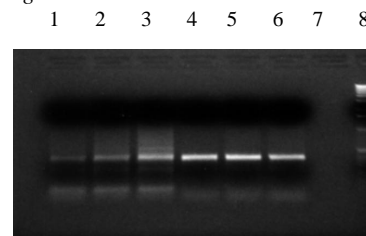
Taq DNA Polymerase from Fisher Scientific: Lanes 1-3, standard PCR; Lanes 4-6, EasyStart PCR; Lane 7, empty; Lane 8, 1kb ladder.

Figure 3



Taq DNA Polymerase from Boehringer Mannheim: Lanes 1-3, standard PCR; Lanes 4-6, EasyStart PCR; Lane 7, empty; Lane 8, 1kb ladder.

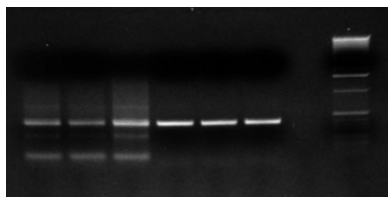
Figure 4



Taq DNA Polymerase from Perkin-Elmer: Lanes 1-3, standard PCR; Lanes 4-6, EasyStart PCR; Lane 7, empty; Lane 8, 1kb ladder.

Figure 5

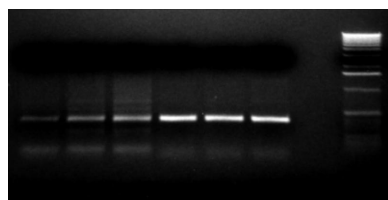
1 2 3 4 5 6 7 8



Taq DNA Polymerase from Stratagene: Lanes 1-3, standard PCR; Lanes 4-6, EasyStart PCR; Lane 7, empty; Lane 8, 1kb ladder.

Figure 6

1 2 3 4 5 6 7 8



Taq DNA Polymerase from Advance Biotechnologies: Lanes 1-3, standard PCR; Lanes 4-6, EasyStart PCR; Lane 7, empty; Lane 8, 1kb ladder.

**REFERENCES**

1. Jindal, et al. 1989. Molecular and Cellular Biology. Vol 9. P2279-2283.
2. Friedman, et al. 1994. Nature Genetics. Vol 8. P399-404.

**TABLE A**

| Manufacturer                             | Enzyme                  | Catalog Numbers  |
|--|-------------------------|--|
| LifeTechnologies (figure 1)              | Taq DNA Polymerase      | 18038-018, 067, 042 (native)<br>10342-053, 020, 046 (recombinant)                    |
| Fisher Scientific (figure 2)             | Taq DNA Polymerase      | FB60045<br>PR-M1661-69   |
| Boehringer Mannheim (figure 3)           | Taq DNA Polymerase      | 1146165, 1146173, 1418432,<br>1596594, 1435094, 1648679,<br>1647687                  |
| Perkin-Elmer (figure 4)                  | AmpliTaq DNA Polymerase | N801-0060, 0101, 0106, 1012,<br>0105, 0107, 0070 (recombinant)<br>N801-0046 (native) |
| Stratagene (figure 5)                    | Taq DNA Polymerase      | 600131   |
| Advanced Biotechnologies - UK (figure 6) | Red Hot DNA Polymerase  | N808-0130  |

For a complete listing of all compatible enzymes see EasyStart protocol.

This product is sold under licensing arrangements with Roche Molecular Systems, Inc., and the Perkin-Elmer Corporation. The purchase price of this product does not include a license under Roche patents covering the performance of the polymerase chain reaction or their foreign counterparts nor under any Roche thermostable polymerase patents or their foreign counterparts. U.S. Patent #5,411,876. MBP is a registered trademark and EasyStart is a trademark of Molecular BioProducts, Inc., San Diego, CA. U.S. Patent #5,576,197. ©MBP 2001.

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