

MBP[®] EasyStart[™] PCR Mix-in-a-Tube Protocol

1.0 Introduction

EasyStart PCR Mix-in-a-Tube, developed by Molecular BioProducts, Inc., represents the next generation in PCR technology. Pre-aliquoted, non-specific reagents have been sealed under a wax barrier, eliminating many of the pipetting steps normally associated with PCR. With this new technology, it is only necessary to add reaction-specific reagents such as DNA polymerase, template DNA and primers, thus reducing the chance of contamination and ensuring more consistent results. **Note: Some DNA polymerases require the addition of Triton[®] X-100 for optimal performance;** please see section 5.7 for more information.

2.0 Important Handling Instructions

EasyStart uses a wax barrier to seal and preserve the integrity of the lower layer reagent mixture. EasyStart is shipped ready-to-use and does not require further sterilization and therefore should not be autoclaved or exposed to heat.

EasyStart PCR tubes are thin-walled to optimize heat transfer and reaction conditions. When handling the tubes, finger pressure can damage the thin walls, so for best results, use MBP's Reversible or FlipStrip[™] Microtube Racks.

EasyStart tubes can be centrifuged by following this simple procedure: Remove the lids from 1.5 ml centrifuge tubes, fill them partially with water (1300 μ l for 0.2 ml tubes and 700 μ l for 0.5 ml tubes) and place sealed EasyStart tubes inside. The water supports the thin-walled tubes during high speed centrifuging.

3.0 Storage Requirements

EasyStart can be stored for extended periods of time at room temperature without affecting the reagent mixture. This is possible because the wax layer forms a hermetic seal which protects the reagents from degradation for at least one year. However, if exposed to high temperatures, the wax layer can soften and potentially expose the reagents if the tubes are not kept in an upright position.

4.0 Controlling Contamination

It is important to recognize that the elevated

sensitivity gained through using EasyStart tubes increases your PCR's detection of not only the target DNA, but also any non-target DNA. Therefore, special attention must be given to contamination control procedures. We suggest the following precautions:

1) Prepare the upper layer reaction mixture under a laminar flow hood.

2) Wear a face mask, hair net and gloves; change gloves frequently.

3) It is possible to minimize the risk of contamination by carefully handling all laboratory supplies and experimental samples. The use of ART[®] self-sealing barrier tips during all phases will eliminate the cross-contamination of samples by aerosol transfer from the pipettor to subsequent samples. ART tips are available pre-sterilized and contain a patented, self-sealing barrier scientifically proven to eliminate aerosol and liquid contamination.

4) To prevent the transfer of DNA and PCR product from one surface to another, we recommend the regular use of MBP's RNase AWAY[®] surface decontaminant to clean the thermal cycler block, counter space and the pipettor shaft. When used as directed, this product will eliminate all RNase, RNA, DNase and DNA activity upon contact.

For product information and a copy of our Contamination Control Manual, call MBP's Customer Service department at (800) 995-2787 in the U.S., (619) 453-7551 outside the U.S., fax to (619) 453-4367, or send e-mail to info@mbpinc.com. You may also visit us on the World Wide Web at <http://www.mbpinc.com>.

5.0 Product Information

5.1 Catalog Information

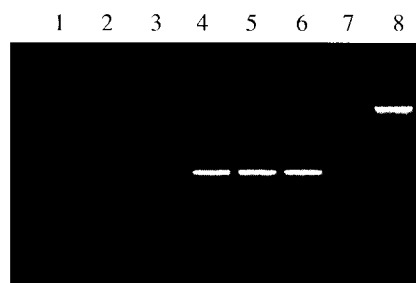
See table 1

5.2 Pre-Added Reagents

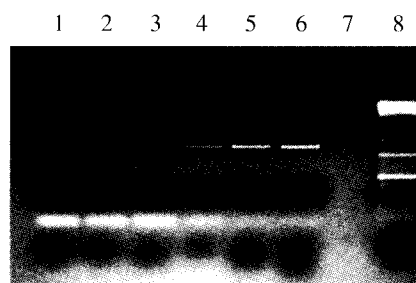
EasyStart contains MgCl₂, dNTPs, dH₂O, and 10X buffer consisting of 200 mM Tris-HCl (pH 8.4) and 500 mM KCl, under the wax layer. (see table 2).

5.3 Optimization

EasyStart has been optimized to provide superior results with a MgCl₂ concentration of 2 mM. With EasyStart's enhanced specificity and yield, this concentration has been shown to be optimal for a variety of reaction conditions, even those that may typically have demanded higher MgCl₂ levels (see gel photos below).



Rat p53 pseudogene (443bp): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder.



Zea Mays polyubiquitin (2.5kb): lanes 1-3, conventional PCR; lanes 4-6 EasyStart PCR; lane 7, empty; lane 8, 1kb ladder.

If greater concentrations are required, MgCl₂ can be added to the upper layer reaction mixture, although this will reduce the benefits of EasyStart's automatic hot start reaction and increase the probability of mis-primers and primer dimerization.

5.4 Preparation of Upper Layer Reaction Mix

See table 3

5.5 Recommended Final Concentrations

See table 4

5.6 Thermal Cycler Compatibility

EasyStart tubes are standard 0.2 ml and 0.5 ml conical, thin-walled PCR tubes designed to fit major-brand thermal cyclers

including those by Perkin-Elmer, MJ Research, Barnstead Thermolyne, Hybaid, Ericomp and Precision.

5.7 Enzyme Compatibility

EasyStart is compatible with a wide variety of enzymes including those from

Boehringer Mannheim, Perkin Elmer, Promega, Life Technologies (Gibco BRL), Fisher Scientific, Advanced Biotechnologies, Finnzymes and Stratagene. Some enzymes have been shown to require the addition of Triton X-100 (included) for optimal performance. If the buffer description included with your enzyme's protocol

lists this ingredient, please see table 3 for instructions on including Triton-X 100 in the upper layer reaction mixture.

Enzyme Compatibility Chart

Company	Enzyme	Catalog Numbers
Advanced Biotechnologies (U.K.)	Red Hot DNA Polymerase	AB-0406
Ambion	SuperTaq	2050, 2052
Boehringer Mannheim	Taq DNA Polymerase	1146165, 1146173, 1418432, 1596594, 1435094, 1647679, 1647687
Finnzymes	DyNAzyme	F-500S, F-500L, F-500L-10, F-500L-20 F-501S, F-501L, F-501L-10, F-501L-20 F-502S, F-502L, F-502L-10, F-502L-20 F-503S, F-503L, F-503L-10, F-503L-20 (Requires Triton X-100)
Fisher Scientific	Taq DNA Polymerase	FB600045 PR-M 1661-69
LifeTechnologies (Gibco BRL)	Taq DNA Polymerase	18038-018, 067, 042 (native) 10342-053, 020, 046 (recombinant)
New England Biolabs	Vent DNA Polymerase	254S, 254L, 257L, 257S (Requires Triton X-100)
Perkin-Elmer	AmpliTaq DNA Polymerase	N801-0060, 0101, 0106, 1012, 0105, 0107, 0070 (recombinant) N801-0046 (native)
Promega	Taq DNA Polymerase in Storage Buffer A	M1861 (Requires Triton X-100)
	Taq DNA Polymerase in Storage Buffer B	M1661
Qiagen	Taq DNA Polymerase	201203, 201205, 201207
Stratagene	Taq DNA Polymerase	600131, 600132, 600139
	Pfu DNA Polymerase	600135, 600136, 600140, 600153, 600154, 600159 (Requires Triton X-100)

6.0 Procedure

6.1 Reaction

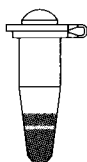
Step One: Assembling the reaction mixture.

Add your PCR reagents including template DNA, primers, enzyme, dH₂O and Triton X-100 (if required) on top of EasyStart's wax layer (see table 3). Assembling a master mix of reagents prior to aliquoting into individual tubes may be preferred when performing multiple reactions.

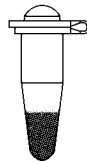
Step Two: Beginning the EasyStart reaction.

Place EasyStart tubes into a thermal cycler and begin PCR. It is important that the first denaturation cycle be between one and five minutes to ensure the thorough mixing of the reaction layers and the complete denaturation of your target. After completing the thermal cycles, PCR product can be removed from the EasyStart tubes by penetrating the wax barrier with a pipet tip.

Step 1



Step 2



6.2 Suggestions

1) Exercise caution when withdrawing the sample; vigorous penetration of the wax layer may result in a spray of the amplified product. Slowly puncture the center of the wax barrier where the meniscus is thinnest and rotate the pipettor in a circular motion to enlarge the hole. It may be useful to penetrate the wax with the pipet tip at a slight angle instead of at 90°.

2) For low-volume EasyStart reactions, sensor tube oil volume should approximate the PCR reaction volume; be sure to use a thin-walled tube.

3) Normal trace amounts of evaporation may be observed (less than 2 µl); this will not affect your PCR.

7.0 Troubleshooting Guidelines

Problem	Probable Cause	Solution
Artifactual Smears	Too much enzyme.	Reduce amount of enzyme.
	Low annealing temperature. GC-rich primers will dimerize at low annealing temperatures.	Increase annealing temperature.
No Amplification	Enzyme may require Triton X-100.	Check manufacturer's buffer reagents and add according to EasyStart protocol.
Extra Bands	Amplified DNA Contaminants. The increased sensitivity of EasyStart will amplify even the smallest quantities of DNA.	Contamination may be present. Use RNase AWAY surface decontaminant and ART self-sealing barrier tips. See EasyStart protocol section 4.0.
No Wax Inversion	Volume of upper reaction layer is too small.	Ensure that upper reaction layer volume is approximately 50% of total reaction volume.
	Initial heating step is too short.	Make sure that the initial heat step is long enough and hot enough to melt the wax barrier.

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EasyStart Tables

table 1

Product	Micro 20	Micro 50	Micro 100	100
Catalog Number	6028	6020	6024	6025
Tube Sizes	0.2 ml	0.2 ml	0.2 ml	0.5 ml
Reaction Volume	20 μ l	50 μ l	100 μ l	100 μ l

table 2

Pre-added Ingredients	Micro 20	Micro 20 Strips	Micro 50	Micro 100	100
50 mM MgCl ₂	0.8 μ l	0.8 μ l	2 μ l	4 μ l	4 μ l
10X PCR Buffer	2 μ l	2 μ l	5 μ l	10 μ l	10 μ l
2.5 mM dNTP Mix	1.6 μ l	1.6 μ l	4 μ l	8 μ l	8 μ l
dH ₂ O	5.6 μ l	5.6 μ l	14 μ l	28 μ l	28 μ l
Total Pre-added Volume	10 μ l	10 μ l	25 μ l	50 μ l	50 μ l

table 3

Upper Layer Reaction Mix	Micro 20	Micro 50	Micro 100	100
Primer 1 (25 M)	0.3 μ l	0.65 μ l	1.25 μ l	1.25 μ l
Primer 2 (25 M)	0.3 μ l	0.65 μ l	1.25 μ l	1.25 μ l
Template DNA	varies	varies	varies	varies
Taq DNA Polymerase (5 U/ l)	0.1 μ l	0.25 μ l	0.5 μ l	0.5 μ l
1% solution Triton X-100 (if required)	2 μ l	5 μ l	10 μ l	10 μ l
dH ₂ O	fill to 10 μ l	fill to 25 μ l	fill to 50 μ l	fill to 50 μ l
Total Volume	10 μ l	25 μ l	50 μ l	50 μ l

table 4

Final Concentrations	Micro 20	Micro 20 Strips	Micro 50	Micro 100	100
MgCl ₂	2 mM	2 mM	2 mM	2 mM	2 mM
PCR Buffer	1X	1X	1X	1X	1X
dNTP Mix	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM
Primer 1	0.37 μ M	0.37 μ M	0.32 μ M	0.31 μ M	0.31 μ M
Primer 2	0.37 μ M	0.37 μ M	0.32 μ M	0.31 μ M	0.31 μ M