Perfect Match PCR Enhancer

Instruction Manual

Catalog #600129 (100 U) and #600130 (200 U)

Revision C.0

For Research Use Only. Not for use in diagnostic procedures.

600129-12

Agilent Technologies

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

Email

techservices@agilent.com

World Wide Web

www.genomics.agilent.com

Telephone

Location	Telephone
United States and Canada	800 227 9770
Austria	01 25125 6800
Benelux	02 404 92 22
Denmark	45 70 13 00 30
Finland	010 802 220
France	0810 446 446
Germany	0800 603 1000
Italy	800 012575
Netherlands	020 547 2600
Spain	901 11 68 90
Sweden	08 506 4 8960
Switzerland	0848 8035 60
UK/Ireland	0845 712 5292
All Other Countries	Please visit *www.genomics.agilent.com* and click **Contact Us**

Perfect Match PCR Enhancer

CONTENTS

Perfect Match PCR Enhancer

MATERIALS PROVIDED

Material provided	Quantity	
	Catalog #600129	**Catalog #600130**
Perfect Match PCR enhancer (1U/µl)	100 U	200 U

STORAGE CONDITIONS

Perfect Match PCR Enhancer: –20°C

Revision C.0

INTRODUCTION

Perfect Match PCR enhancer* is an additive that increases the specificity and yield of primary PCR amplification products. When added to genomic in vitro amplification reactions, Perfect Match PCR enhancer destabilizes many mismatched primer-template complexes that would otherwise result in a heterogeneous population of amplified molecules. Perfect Match PCR enhancer is an effective solution for the problem of PCR artifacts generated by false priming by removing secondary structure for reactions present within the target sequence.

The benefits derived from Perfect Match PCR enhancer are dependent on the degree of primer-template homology. For example, primer-template complexes that contain substantial mismatched nucleotides at or near the 3´ terminus of the primer are destabilized by Perfect Match PCR enhancer and will not generate amplified products. However, most perfect or near-perfect primer-template complexes or primers with regions of nonhomology at the 5´ terminus produce equal or greater amounts of amplified product when using Perfect Match PCR enhancer. Templates between 2000 and 5000 bp may also be successfully amplified using Perfect Match PCR enhancer.

OPTIMIZATION OF PERFECT MATCH PCR ENHANCER

In-house studies indicate that Perfect Match PCR enhancer increases primer extension reaction product specificity in genomic and plasmid DNA amplifications. Excellent product yield may be obtained by optimizing the ratio of Perfect Match PCR enhancer to DNA template.

- For plasmid templates, use 1 U of Perfect Match PCR enhancer/µg of DNA template.

  Note *Linearized plasmid DNA works better than supercoiled DNA. Carrier genomic DNA may improve plasmid primer extension reaction amplifications.*

- For genomic templates and templates >2000 bp, use 1 U of Perfect Match PCR enhancer/100 ng of DNA template.

PRIMER SPECIFICATIONS

When using Perfect Match PCR enhancer in genomic amplification reactions, primers >16 mers are recommended. If using primers <17 mers, the annealing temperature should be reduced.

Perfect Match PCR enhancer can work with nested primers and degenerate primer extension, if the desired product molecules have substantial complementarity with the 3´-terminal nucleotides of the primers.

*U.S. Patent Nos. 5,773,257, 5,646,019, 5,605,824 and 5,449,603.

AMPLIFICATION PROTOCOL

Note *The following protocol is designed for one primer extension amplification reaction.*

1. To prepare the amplification reaction, add the following components to a 0.5-ml microcentrifuge tube:

   80 µl of distilled water (dH_2O)
   10 µl of *Taq* DNA polymerase 10× reaction buffer
   0.8 µl of 25 mM dNTP mix
   1 µl of DNA template (100 ng/µl)
   1 µl of primer #1 (250 ng/µl)
   1 µl of primer #2 (250 ng/µl)

   Gently mix the amplification reaction to adequately disperse the genomic DNA.

2. Transfer the microcentrifuge tube to the thermal cycler and premelt the amplification reaction by incubating the tube for 5 minutes at 95°C.

3. Following the premelt step, program the thermal cycler to the appropriate annealing temperature (37–60°C). Incubate the microcentrifuge tube for 5 minutes.

   Note *Perform the following steps as quickly as possible. Maintaining the primer extension reaction cocktail for prolonged periods at room temperature can result in an increase in false-priming events, which may lead to a decrease in product specificity.*

4. Remove the microcentrifuge tube from the thermal cycler and pulse spin the tube in a microcentrifuge.

5. Add 1 U of Perfect Match PCR enhancer/100 ng of DNA template. If amplifying plasmid DNA, use 1 U of Perfect Match enhancer PCR /µg of DNA template. Then add 2.5 U of *Taq* DNA polymerase to the microcentrifuge tube. A cocktail of Perfect Match PCR enhancer and *Taq* DNA polymerase may be prepared if desired.

6. Overlay the amplification reaction with 50 µl (~2 drops) of mineral oil and transfer the microcentrifuge tube to the thermal cycler.

7. Program the thermal cycler for 30 cycles using the cycling parameters indicated in the following table:

Cycle	Temperature	Duration
Extension	72°C	30–60 seconds/kb
Melting	94–95°C	1 minute
Annealing	37–60°C	2–3 minutes

Follow the final cycle with a 10-minute extension at 72°C.

TROUBLESHOOTING

Perfect Match PCR enhancer increases the relative binding energy of matched primer-template combinations; therefore, varying the temperatures and times of the primer extension reaction cycles may substantially increase the benefits obtained with Perfect Match PCR enhancer.

Observation	Suggestion
No product or low yield	Decrease the annealing temperature by several degrees (5–10°C) and increase the duration of the annealing and extension cycles by up to 50%
False products (background)	Increase the annealing temperature by several degrees (5–10°C) and decrease the duration of the annealing and extension cycles by up to 50%

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.