Taq Extender PCR Additive

Instruction Manual

Catalog #600148 (1000 U)

Revision D0

Laboratory Reagent.

600148-12

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Taq Extender PCR Additive

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MATERIALS PROVIDED

Materials provided	Quantity
Taq Extender PCR additive (5 U/µl)	200 µl
Taq Extender 10× reaction buffer[a]	1 ml

[a] See *Preparation of Reagents*.

STORAGE CONDITIONS

All components: –20°C

ADDITIONAL MATERIALS REQUIRED

Taq DNA polymerase

Revision D0

INTRODUCTION

Taq Extender PCR additive is a polymerase enhancer that improves the reliability and yield of conventional polymerase chain reaction (PCR) amplifications. This additive increases the efficiency at which *Taq* DNA polymerase performs extension reactions on specific DNA segments in each cycle of PCR, thus resulting in a greater percentage of the extension reactions reaching completion. In addition, *Taq* Extender PCR additive improves the PCR amplification of difficult templates and increases the reliability and yield of many PCR targets up to 10 kb in length.[1] The easy-to-use *Taq* Extender PCR additive is simply added to amplification reactions in an amount equal to that of *Taq* DNA polymerase, the standard *Taq* 10× reaction buffer is replaced with an optimized *Taq* Extender 10× reaction buffer and cycling is performed using standard PCR conditions.

PROTOCOL

1. Prepare the amplification reaction using a standard PCR protocol and implement the modifications to the standard protocol as described in step 2. Determine the standard reaction parameters, such as the deoxynucleotide (dNTP) concentration, the amount of *Taq* DNA polymerase required and the cycling conditions (see step 3).

2. Modify the standard PCR protocol as outlined below.

   Notes *Modifications of primer length or cycling temperatures are not necessary.*

   Use of thin-wall microcentrifuge tubes is highly recommended, but not critical, to improve the heat transfer, which will further enhance the efficacy of the Taq *Extender PCR additive.*

   a. Substitute the standard *Taq* 10× reaction buffer with the *Taq* Extender 10× reaction buffer.

   b. Add an equal number of units of *Taq* Extender PCR additive and *Taq* DNA polymerase to the amplification reaction [i.e., add 1 µl of *Taq* Extender PCR additive (5 U/µl) to an amplification reaction requiring 1 µl of *Taq* DNA polymerase (5 U/µl)]. Use a minimum of 1 U each of *Taq* DNA polymerase and *Taq* Extender PCR additive per kilobase to be amplified.

3. Perform the amplification reaction using standard cycling conditions, allowing at least 30–60 seconds of extension time for each kilobase to be amplified.[2]

TROUBLESHOOTING

Observation	Solution(s)
Low yield	Minor lot-to-lot variations in the concentration of *Taq* DNA polymerases from various manufacturers may affect PCR product yields and may necessitate the use of greater or lesser amounts of *Taq* Extender PCR additive per amplification reaction in order to achieve optimal PCR results
	Choice of the *Taq* Extender 10× reaction buffer may also affect PCR product yields. If the *Taq* Extender 10× reaction buffer provided with this kit does not appear to be optimized for your particular amplification system, further buffer optimization may be required
	Insufficient amounts of *Taq* DNA polymerase or inadequate extension times may also contribute to poor PCR product yields. Optimal PCR product yields are obtained by using a minimum of 1 U of *Taq* DNA polymerase per kilobase to be amplified and by allowing at least 30–60 seconds of extension time for each kilobase to be amplified[2]
Artifactual smears	PCR smearing may be due primarily to minor contaminants of the *Taq* DNA polymerase. Two possible solutions are to use a different lot number of the enzyme or to reduce the extension time

PREPARATION OF REAGENTS

Taq Extender 10× Reaction Buffer

200 mM Tris-HCl (pH 8.8)
100 mM KCl
100 mM $(NH_4)_2SO_4$
20 mM $MgSO_4$
1% Triton X-100
1 mg/ml nuclease-free bovine serum albumin (BSA)

REFERENCES

1. Nielson, K., Schoettlin, W., Bauer, J. C. and Mathur, E. (1994) *Strategies* 7(2):27.
2. Innis, M. A., Myambo, K. B., Gelfand, D. H. and Brow, M. A. (1988) *Proc Natl Acad Sci U S A* 85(24):9436-40.

MSDS INFORMATION

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