



CERTIFICATE OF ANALYSIS

Long PCR Control Set

#K0201 for 25 reactions

Lot:

Store at -20°C

Description

The Long PCR Control set is designed for testing of optimal long PCR cycling conditions and for quality control of Long PCR enzyme Mix.

The set contains λ DNA, direct and two reverse primers for amplification of 20kb and 30kb PCR fragments with the Long PCR Enzyme Mix. At concentration of $4\mu\text{M}$ supplied, $5\mu\text{l}$ of primer in a $50\mu\text{l}$ PCR reaction mixture will result in a primer concentration of 400nM.

A detailed Long PCR protocol is available with the [Long PCR enzyme Mix \(#K0181, #K0182\)](#). Good PCR results are achieved when annealing and extension are merged into one step and performed for 14 minutes and 20 minutes at 68°C for amplification of 20kb and 30kb fragments, respectively.

Components

1. Direct Long PCR primer

250µl of direct Long PCR primer in deionized water, 4µM,
sequence:

5'- CTG ATG AGT TCG TGT CCG TAC AAC TGG CGT AAT C- 3'

2. Reverse 20kb PCR primer

125µl of Reverse 20kb PCR primer in deionized water, 4µM,
sequence:

5'- GTG CAC CAT GCA ACA TGA ATA ACA GTG GGT TAT C- 3'

3. Reverse 30kb PCR primer

125µl of Reverse 30kb PCR primer in deionized water, 4µM,
sequence:

5'- GAA AGT TAT CGC TAG TCA GTG GCC TGA AGA GAC G- 3'

4. Lambda DNA

100µl of lambda DNA in TE buffer, 2.5ng/µl

Preparation of Reaction Mixture

Always set up the PCR on ice. Setting up of PCR at room temperature can result in a primer degradation by the 3' → 5' exonuclease proofreading activity of the enzyme mix.

Gently vortex and briefly centrifuge all solutions after thawing.

To perform several parallel reactions, prepare a master mix containing water, buffer, dNTPs, primers and template DNA solutions in a single tube, which can then be aliquoted into individual tubes. The Long PCR Enzyme Mix should be added last. This method of setting reactions minimizes a possibility of pipetting errors and saves time by reducing a number of reagent transfers.

Add, in a thin walled PCR tube, on ice:

for synthesis of 20kb PCR fragment

Reagent	Quantity for 50µl	Final concentration
Sterile deionized H ₂ O	29µl	–
10X Long PCR buffer with MgCl ₂	5µl	1X
2mM dNTP mix	5µl	0.2mM of each
Direct Long PCR primer	5µl	0.4µM
Reverse 20kb PCR primer	5µl	0.4µM
Lambda DNA, 2.5ng/µl	0.5µl	1.25ng/50µl
Long PCR enzyme Mix	0.5µl	2.5u/50µl

Recommended cycling conditions

Segment	Temperature	Duration	Number of cycles
Initial denaturation	94°C	2 minutes	1
Denaturation	94°C*	20 seconds	10
Annealing-elongation	68°C	14 minutes	
Denaturation	94°C*	20 seconds	15
Annealing-elongation	68°C	14 minutes + 10 seconds /cycle	
Final elongation	68°C	10 minutes	1

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for synthesis of 30kb PCR fragment

Reagent	Quantity for 50µl	Final concentration
Sterile deionized H ₂ O	27µl	–
10X Long PCR buffer with MgCl ₂	5µl	1X
2mM dNTP mix	5µl	0.2mM of each
Direct Long PCR primer	5µl	0.4µM
Reverse 30kb PCR primer	5µl	0.4µM
DMSO	2µl	4%
Lambda DNA, 2.5ng/µl	0.5µl	1.25ng/50µl
Long PCR enzyme Mix	0.5µl	2.5u/50µl

Recommended cycling conditions

Segment	Temperature	Duration	Number of cycles
Initial denaturation	94°C	2 minutes	1
Denaturation	94°C*	20 seconds	10
Annealing-elongation	68°C	20 minutes	
Denaturation	94°C*	20 seconds	15
Annealing-elongation	68°C	20 minutes + 15 seconds /cycle	
Final elongation	68°C	10 minutes	1

*Denaturation time is very dependent on the PCR tubes and thermal cycler used. Thin-wall type PCR tubes are recommended. The

optimal denaturation conditions for Applied Biosystems GeneAmp® 9700 Thermocycler are 94°C for 10sec, for Eppendorf Mastercycler® - 95°C for 15sec, for Perkin Elmer 480 - 94°C for 20sec. When using other thermocyclers the denaturation conditions have to be adjusted.

For visualization on 0.6-0.7% agarose gel load 10µl of PCR reaction mixture per 4mm gel lane.

Trademarks

GeneAmp is a registered trademark of Applied Biosystems. **Eppendorf Mastercycler** is a registered trademark of Eppendorf.

QUALITY CONTROL

Each lot of Long PCR Control Set is tested in PCR for synthesis of 20kb and 30kb DNA fragments using Long PCR Enzyme Mix.

Quality authorized by:



Jurgita Zilinskiene

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

Updated March 12, 2002