LabQ Taq DNA Polymerase

SHIPPING: on dry ice / blue ice

LOT: see vial

PACK SIZES

LQ-92TDP500U: 500 Units LabQ DNA Polymerase

KIT COMPONENTS

500 U LabQ DNA Polymerase (5 U / µl)
2x 1.5 ml 10X LabQ Buffer clear

ADDITIONAL MATERIALS REQUIRED

Nuclease free dH_2O
Nuclease free PCR tubes or plates
Thermocycler
PCR Primer (10 µM each)
dNTP Mix (10 mM each)

STORAGE & STABILITY

Products are shipped on dry ice / blue ice. All kit components should be stored at −20°C upon arrival. Excessive freeze/thawing is not recommended. When stored under recommended conditions, full activity of the kit is retained until the expiry date – see product label.
Excessive freeze / thawing should be avoided, protect from light.

NOTE: for research use only

PRODUCT DESCRIPTION

LabQ® DNA Polymerase is a Taq DNA polymerase suitable for all common PCR applications. It is supplied in a formulation containing 50% glycerol and buffers designed for optimall amplification.

All LabQ® Polymerase Kits come with 10X LabQ Buffer Clear, 5X LabQ Buffer Gold can be purchased separately (Cat. # PCCVTBGOLD)

The 5X LabQ Buffer Gold contains a yellow dye that co-migrates with the running front during separation on an agarose gel and therefore helps to monitor the progression of the electrophoresis. In a 1% agarose gel, the yellow dye runs below 50 bp. The 5X LabQ Buffer Gold also leads to increased density of the sample. Therefore, the addition of a separate loading dye can be omitted.

If the sample will be used directly for fluorescence and absorbance readings without previous purification, 10X LabQ Buffer Clear should be used as the yellow dye may interfere with these measurements.
All buffers contain $MgCl_2$ at a concentration that results in a final concentration of 1.5 mM in the 1X reaction.

Biological Source

LabQ® DNA Polymerase is recombinantly produced in bacteria.

Enzyme Concentration

5 U/µl.

Storage Conditions

Storage conditions for the individual components are stated on the product labels.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C. The reaction conditions are specified below under Standard DNA Polymerase Assay Conditions.

Mutation Rate

2.28×10^{-5}

Optimization Parameters

LabQ® DNA polymerase is a robust, all-round polymerase that can be used for all common PCR applications. However, for optimal amplification yield and specificity, it may be necessary to optimize the reaction parameters given in this manual. These parameters include: the concentration and purity of the used template and primers, the amount of enzyme, cycling parameters and primer design.

Primer and Template

For use with LabQ® DNA Polymerase, the use of desalted primers und purified template DNA is strongly recommended to ensure the absence of extraneous salts that might interfere with the reaction. Higher purity in template and primers may result in improved results.

The optimum amount of template DNA depends strongly on the type of template; in general, no more than 1 µg of DNA on a 50 µl reaction should be used. For single-copy targets, 10-50 ng of template in a 50 µl reaction are typically used. This amount can be reduced when amplifying multicopy targets e.g. from plasmid DNA. In this case, 5 ng to 25 ng are recommended.

When using fewer cycles to reduce the probability of PCR-introduced mutations, keep in mind to use an adequately increased amount of template DNA to ensure a comparable yield of PCR product.

We recommend using primers at a final concentration of 0.1–0.5 µM (~50–125 ng of an 18- to 25-mer oligonucleotide primer / 50 µl reaction).

Enzyme Concentration

The amount of LabQ® DNA polymerase necessary for optimal amplification and specificity depends on the individual target system to be amplified. For most applications, however, 1 U of enzyme in a 50 µl reaction is used.

Cycling Parameters

The cycling parameters are a crucial step for optimization as they are highly dependent on the individual properties of the target to be amplified.

Denaturation Conditions

Long exposure to high temperatures could damage the template DNA, so the denaturation temperature should be as low as possible. We recommend a denaturation temperature of 94°C.

EXTENSION TIME & TEMPERATURE

We recommend an extension temperature of 72°C and 1 minute extension time for 1 kb template size. Longer extension time may improve the yield for difficult targets.

Primer Design

Choose primer pairs that exhibit similar melting temperatures to ensure specific amplification of your target. Primer sequences should furthermore be analyzed for hairpin or duplex formation as well as other binding sites on the target. Typically, melting temperatures between 52°C and 60°C are used; higher melting temperatures may pose a problem in the extension step and are not recommended.

Reaction Buffer

Make sure to choose the right buffer for your application: *10X LabQ Buffer Clear* can be used for any application, *5X LabQ Buffer Gold* makes the PCR reaction ready-to-load after amplification but may interfere with fluorescence and absorbance readings.
It may also improve the reaction to increase the concentration of $MgCl_2$ up to 5 mM. Higher concentrations though may lead to decreased target specificity.

PCR PROTOCOL Using LabQ® DNA Polymerase

Setting Up the Reaction

- Before setting up the reactions, please review *Optimization Parameters.* Before use, thaw all components completely and mix gently. Prepare the reaction on ice in a sterile, nuclease free tube and mix gently after addition of the polymerase. Collect all liquid at the bottom of the tube by a quick spin.
- Table 2. provides conditions for a standard PCR reaction. The recipe is for one
- reaction and must be upscaled for multiple samples.

Component	Volume	Final Concentration
Reaction Buffer[1]	5 µl	1X
dNTP Mix (10 mM each)[1]	0.5 µl	0.1 mM
Primer 1(10 µM)	1 µl	0.1 µM – 0.5 µM
Primer 2 (10 µM)	1 µl	0.1 µM – 0.5 µM
LabQ® DNA Polymerase (5 U/µL)	0.2 µl	1 U
template DNA	1 µl	<1 µg
dH_2O	to 50 µl[2]	

[1]Thaw completely, and vortex thoroughly prior to use. Adjust volume when using *5X LabQ Buffer Gold*

[2] Adjust volume when using *5X LabQ Buffer Gold*

- Keep the reactions on ice until transfer to the thermocycler, then cycle according to the guidelines in Table 3.

Step	Cycles	Temperature	Duration
Initial Denaturation	1	94°C	5 minute
Amplification	25-35	94°C	30 seconds
		T_m – 5°C	30 seconds
		72°C	1 minute / kb
Final Extension	1	72°C	5 minutes
Hold	1	4°C	

Evaluation of the PCR Reaction Using Gel Electrophoresis

Analyze the amplification reaction by gel electrophoresis using an acrylamide or agarose gel of appropriate percentage.

Troubleshooting

Problem	Suggestion
No PCR product or low yield	Ensure that a sufficient amount of template DNA was used and check the quality of the template DNA on an agarose gel.
	Reaction parameters may require optimization: add 0.25 U more of LabQ DNA polymerase / 50 µl reaction or try increasing the $MgCl_2$ concentration in 0.2 mM steps.
	Optimize the annealing temperature by lowering it in 2°C steps.
	Optimize the extension time in 30 second steps, especially if your target is ≥1 kb.
	Be sure to use the supplied *10X LabQ Buffer.*
	Test different primer : template ratios.
	Make sure your primers anneal to your target sequence and have matching T_ms.
	Dilute your template DNA to dilute potentially inhibiting components.
	Try adding PCR additives like DMSO, betaine or BSA.
Reaction yields multiple bands	Optimize primer annealing temperatures by raising the temperature in 2°C steps.
	Make sure your primers have only one binding site on your template.
	Analyze the primers to exclude hairpin or duplex formation.
Amplification product appears as a smear after gel electrophoresis	Use clean pipettes, nuclease-free plastics and fresh solutions when setting upt the reactions.
	Optimize denaturation time by increasing duration in increments of 30 seconds.
	Optimize denaturation temperature by increasing in 1°C increments.

References

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5. Rychlik, W., Spencer, W. J. and Rhoads, R. E. (1990) Nucleic Acids Res 18(21):6409-12.
6. Chien, A., Edgar, D.B. and Trela, J.M. (1976). J. Bact.. 127, 1550-1557.
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SAFETY PRECAUTIONS

For safety instructions please have a look at the Material Safety Data Sheets (MSDS) under: www.labconsulting.at

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