Ampliqon

AmpliQ Genomic Amplifier Kit

Cat. No.: 280801 (25 reactions)

Cat. No.	Size Reactions	5X Amplifier Buffer (dNTP included)	10X Primer-mix	Amplifier Polymerase	Control Genomic DNA (10 ng/µl)
280801	25	250 µL	50 µL	25 µL	25 µL
280803	100	1 mL	200 µL	100 µL	100 µL
280805	500	4x 1.25 mL	1 mL	500 µL	500 µL

Storage: Store the kit at -20°C up to two months.

Note: For longer storage, the Amplifier polymerase should be stored at –70 °C; all other component can be stored at –20 °C

Reagent for in-vitro laboratory use only

General Description

The AmpliQ Genomic Amplifier Kit generates an almost unlimited source of DNA for genetic studies. The AmpliQ Genomic Amplifier method exponentially amplifies single or double stranded linear DNA template during an isothermal strand displacement reaction. Amplification of genomic DNA from lysates generates representative, high fidelity DNA (error rate 10^7) that can be used in various genetic studies. The DNA generated by the AmpliQ Genomic Amplifier Kit is high molecular weight and double-stranded, however a small part of the DNA will be single stranded. Most DNA purification method can be used to generate starting template. In many cases unpurified cell lysates can be directly used as starting material. Typically DNA in the µg range is produced from ng starting material in an overnight incubation at 30°C.

Key Features

- Getting unlimited test material from limited sources of DNA material.
- From ng template DNA to µg DNA in an overnight incubation.
- High-quality and representative DNA for genetic analysis, DNA storing and forensic work.

5X Amplifier buffer

Tris-HCl, pH 7.5, $(NH_4)_2S0_4$, $MgCl_2$, DTT and dNTPs.

Genomic Control DNA

Human genomic DNA 10 ng/ml

Primer-mix

Hexamers primer-mix for random annealing at 30°C

Amplifier Polymerase

Enzyme is supplied in 50 mM Tris-HCl pH 7.5, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% NP40, 50% glycerol.

Quality Control

10 ng of Human genomic DNA will produce between 2-5 ug of DNA. The quality of DNA is judged visually from running the DNA on agarose gel electrophoreses. The quality of the DNA is analysed by Real Time PCR using different primer sets specific for loci on different chromosomes.

Important notes:

- 1 ng (350 copies of human genomic DNA) of DNA is required for efficient representative amplification.
- The AmpliQ Genomic Amplifier Kit is extremely sensitive, very small amounts of any input DNA will be efficiently amplified, it is therefore important to use clean implements and containers.
- It is recommended to use as small volume template DNA as possible (1-2 µL) since contaminants within the sample can inhibit the reaction (i.e. SDS, EDTA, hemoglobin, high salt).
- It is recommended to use as intact DNA as possible, since nicked, “old” or restriction enzyme digested DNA will perform poorly in the reaction.
- In absence of template DNA, an amplified non-specific product will likely appear (hexamer primer amplification), however this product will not influence in later applications.
- It should be tested if the various templates can be applied directly or a DNA purification step has to be performed.
- Some applications are sensitive to residual components of the reaction or carry-over from the starting sample. The need for purifying DNA for downstream applications is best determined empirically.
- For some application an extra 3 minutes denaturation step of the template DNA can help to generate more yield.

Suggested Protocol for the AmpliQ Genomic Amplifier Kit.

This protocol serves as a guideline for DNA amplification. Optimal reaction conditions such as template pre-purification and amount of template DNA may vary and must be individually determined.

1. Thaw 10X Amplifier buffer, Genomic Control DNA and Primer-mix solutions. **It is important to mix the solutions completely before use to avoid localized concentrations of salts**.
2. Prepare a master mix according to Table 1.
3. Use 1 µL of control DNA (10 ng/µL) and run this reaction parallel as a positive control.

Table 1. *Reaction components (master mix and template DNA)*

Component	Vol./reaction	Final Conc.
5X Amplifier buffer	4 µL	1X
Primer-mix	2 µL	1X
Template DNA	Variable	ng range
Distilled water	Variable	1X
Amplifier polymerase	1 µL	-------
Total volume	20 µL	- - - -

4. Mix the master mix thoroughly and incubate at 30°C overnight (8 – 18 hours). A thermal cycler can be used as an incubator.

5. Heat inactivate the amplifier mix by incubating at 65°C for 10 minutes.

6. The tube should now contain amplified DNA that can be used for many applications directly or after a purification step.
   To verify the amplification run a small part of the sample on an agarose gel as illustrated in figure 1.

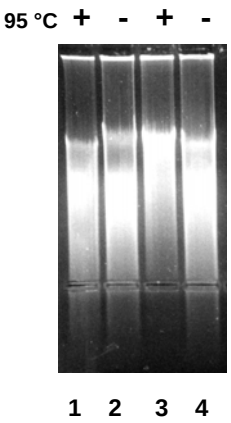

***Figure 1*. Amplification of Human genomic DNA with and without heat denaturation.**
Under standard amplification conditions as described in this datasheet human genomic DNA was amplified overnight using the AmpliQ Genomic Amplifier Kit.

Lane 1 1 ng human genomic DNA with a 95°C template denaturation step.
Lane 2 1 ng human genomic DNA without a 95°C template denaturation step.
Lane 3 10 ng human genomic DNA with a 95°C template denaturation step.
Lane 4 10 ng human genomic DNA without a 95°C template denaturation step.

Quantification of Amplified DNA products

The amount of amplified product can be determined using standard UV absorption (OD_{260}). However since the present of polymerase, dNTP and primers will generate inaccurate results, it is recommended to purify the DNA product before using UV absorption (OD_{260}) method. Standard ethanol precipitation purification is sufficient to solve this issue.

Related Products

Description	Cat. No.
Taq DNA Polymerase (500 Units) *with 10X Ammonium Reaction Buffer* *with 10X Standard Reaction Buffer*	**110303**
Taq DNA Polymerase (500 Units) *with 10X Combination Buffer*	**110403**
Taq DNA Polymerase (500 Units) *with 10X Mg^{++} Free Ammonium Buffer*	**110503**
Taq DNA Polymerase 2.0X Master Mix (100 Reac) *with 2.0 mM MgCl2*	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 Reac) *with 1.5 mM MgCl2,*	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) *with 2.0 mM MgCl2*	**190301**
AccuPOL DNA Polymerase (500 Units)	**210303**
TEMPase Hot Start DNA Polymerase (500Units) *with 10X TEMPase Buffer I* *with 10X TEMPase Buffer II*	**220303**
UniPOL –Long Range PCR (100 Reac)	**270701**
Rapid Ligation Kit (50 React)	**750300**
RT-PCR One Tube (100 Reac)	**740301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer I* (100 Reac)	**230301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer II* (100 Reac)	**230701**
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	**501004**
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	**502004**
GC5 Value Efficiency, 10^8 Cfu/µg pUC19 Chemically Competent Cells, (10x 200µl)	**812010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (10x 50µl)	**805010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (5x 200µl)	**802005**
SuperPath GC10, 10^{10} Cfu/µg pUC19 ElectroCompetent Cells, (5x 80µl)	**830805**
SOC Medium, 10x 10mL	**800000**

NOTICE
In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.