

## AmpliQ Genomic Amplifier

Catalogue no.: 280805 (500 reactions)

Cat. no.	Size Reactions	5 x Amplifier Buffer (dNTP included)	10 x 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,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{MgCl}_2$ , 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	- - - -

- Mix the master mix thoroughly and incubate at 30°C overnight (8 – 18 hours). A thermal cycler can be used as an incubator.
- Heat inactivate the amplifier mix by incubating at 65°C for 10 minutes.
- The tube should now contain amplified DNA that can be used for many applications directly or after a purification step.

**Quantification of Amplified DNA products**

The amount of amplified product can be determined using standard UV absorption (OD<sub>260</sub>). 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<sub>260</sub>) method. Standard ethanol precipitation purification is sufficient to solve this issue.

**Related Products**

Description	Cat. No.
Taq DNA Polymerase, glycerol free (500 units)	<b>100103</b>
Taq DNA Polymerase (500 units) with 10X Ammonium Reaction Buffer with 10X Standard Reaction Buffer	<b>110303</b>
Taq DNA Polymerase (500 units) with 10X Combination Buffer	<b>110403</b>
Taq DNA Polymerase (500 units) with 10X Mg <sup>++</sup> Free Ammonium Buffer	<b>110503</b>
Taq DNA Polymerase 2.0X Master Mix (100 r) with 2.0 mM MgCl <sub>2</sub>	<b>150301</b>
Taq DNA Polymerase 2,0X MaMi RED (100 r) with 1.5 mM MgCl <sub>2</sub> ,	<b>180301</b>
Taq DNA Polymerase 2.0X MaMi RED (100 r) with 2.0 mM MgCl <sub>2</sub>	<b>190301</b>
AccuPOL DNA Polymerase (500 units)	<b>210303</b>
TEMPase Hot Start DNA Polymerase (500 units) with 10X TEMPase Buffer I with 10X TEMPase Buffer II	<b>220303</b>
TEMPase Hot Start 2X Master Mix with TEMPase Buffer I (100 r)	<b>230301</b>
TEMPase Hot Start 2 x Master Mix Blue With Buffer I and II (100 r)	<b>290401</b>
TEMPase Hot Start 2X Master Mix with TEMPase Buffer II (100 reaction)	<b>230701</b>
RealQ PCR 2 x Master Mix (200 reaction) For probe	<b>250407</b>
RealQ PCR 2 x Master Mix (200 reaction) With green dye	<b>250507</b>
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	<b>501004</b>
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	<b>502004</b>

(other product sizes available)

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**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.

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