GRS Taq DNA Polymerase

#GE01.0500 | # GE01.2500 | # GE11.0500 | # GE11.2500 | # GE11.5000

(for research only)

Product: GRS Taq DNA polymerase is a recombinant thermostable enzyme (94kDa monomer) with identical characteristics as native Taq regarding activity, specificity, thermostability, and performance in PCR. GRS Taq possesses 5´→3´ polymerase activity as well as weak 5´→3´ exonuclease activity. The enzyme has no 3´→5´ exonuclease activity (thus no proofreading). GRS Taq is suitable for amplification of targets up to 5kb and is capable of incorporating modified nucleotides (e.g. fluorescent labeled nucleotides). GRS Taq produces 3´ A-overhangs allowing TA-cloning.

Source: Purified from *E.coli* harbouring the DNA polymerase gene from *Thermus aquaticus* YT-1.

Quantity: **#GE01.0500** and **#GE11.0500** comprises 1 vial of 500U of GRS Taq, **#GE01.2500** and **#GE11.2500** comprises 5 vials of 500U of GRS Taq, and **#GE11.5000** comprises 10 vials of 500U of GRS Taq at a concentration of 5U/µl in 20mM Tris-HCl (pH 8.0), containing 0.1mM EDTA, 10mM ß-mercaptoethanol, 222mM ammonium sulphate, 0.5% (v/v) Triton X-100, 0.5% (v/v) Tween®20, and 50% glycerol.

Contents: GRS Taq is supplied with PCR Buffer (10x concentrated), consisting of 500mM Tris-HCl (pH 9.4), 1% Tween®20 and 150mM ammonium sulphate, and with separate 25mM $MgCl_2$.

#GE11.0500, **#GE11.2500** and #**GE11.5000** are supplied with separated dNTPs.

Product	GE01.0500	GE01.2500	GE11.0500	GE11.2500	GE11.5000
GRS Taq (5U/µl)	100µl	5x 100µl	100µl	5x 100µl	10x 100µl
PCR Buffer (10x)	1,5ml	5x 1,5ml	1,5ml	5x 1,5ml	10x 1,5ml
$MgCl_2$ (25mM)	1,5ml	5x 1,5ml	1,5ml	5x 1,5ml	10x 1,5ml
dNTPs (10mM each)	-	-	1,0ml	3x 1,0ml	6x 1,0ml

Applications: Routine PCR

Properties:

Amplicon size: up to 5kb.
Extension Rate 1kb/min
Hotstart: No
A-overhang: Yes

QC: Functionally tested in PCR. Bacterial DNA free (tested by PCR). Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays.

Storage: -20ºC for at least 1 year.

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Basic Protocol

Optimal PCR cycling conditions (incubation times, temperatures, and concentrations) depend on DNA target (GC-content, size, quantity, purity, etc), specific primers, and buffer composition, $MgCl_2$-concentration, and GRS Taq-concentration and need to be determined case by case. We suggest to start with the following basic protocol and subsequently optimize $MgCl_2$-concentration, annealing temperature, incubation times and cycling number.

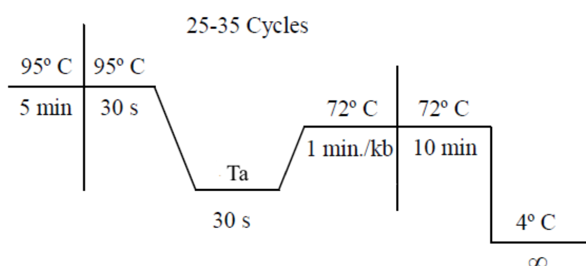
Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Taq:

Component	Volume	Final Conc.
$MgCl_2$ (25mM)	1.5 µl	1.5 mM
dNTPs (10 mM each)	1 µl	0.4 mM
Forward primer (5 pmol/µl)	1.5 µl	0.3 µM
Reverse primer (5 pmol/µl)	1.5 µl	0.3 µM
PCR Buffer (10x)	2.5 µl	1X
Template DNA	0.25 – 10 µl	2-300 ng*
GRS Taq DNA Polymerase (5U/µl)	0.1 -0.5 µl	0.5 – 2.5 U
PCR –grade water	up to 25 µl	

*) In case of plasmid DNA use 1-3 ng, in case of gDNA add up to 100-300ng
For smaller/larger reaction volumes, scale it down/up proportionally.

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control for the detection of possible contaminants, by mixing all components (N+1), except template DNA, dividing the mixture equally into each tube and then add template DNA or PCR grade water in case of the control to the individual PCR tubes.

Set-up PCR amplification as follows:



After an initial cycle of 5 min at 95ºC (complete denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 25-35 times for 30 seconds at 95ºC, 30 seconds at Ta and 1 min (per kb of target DNA) at 72ºC for extension. Set the annealing temperature (Ta) as the melting temperature (Tm) of the primer with the lowest Tm. After amplification, include a final extension step of 10 min at 72ºC to ensure that all amplicons are fully extended and include 3´-A-overhang. Analyze PCR products by DNA Agarose gel electrophoresis.

Optimization

Annealing Temperature (Ta)

Optimizing the annealing temperature is crucial, as a too low temperature might result in non-specific amplification whereas a too high temperature results in no amplification. The melting temperature (Tm) is defined as the temperature in which 50% of the primer and its complementary sequence of the target DNA are present as duplex DNA. By increasing the temperature above the melting temperature, this percentage decreases, however, primers will still anneal (up to a certain point) and initiate extension. PCR can therefore be performed at temperatures of several degrees higher than Tm and it is therefore recommended to optimize the Ta by performing a temperature gradient.

$MgCl_2$

GRS Taq DNA Polymerase is supplied with a PCR Buffer (10x) without magnesium and a separated vial of 25mM $MgCl_2$. Taq requires $MgCl_2$, however, the final concentration may have great influence on both quantity and specificity of the amplification and we highly recommend to optimize the final $MgCl_2$-concentration (normally between 1 and 3mM in steps of 0.5mM).

Incubation times

Denaturation and annealing steps may require less time depending on the thermocycler apparatus, reaction volume and PCR tube (varies with the efficiency of heat-transfer). It might be worthwhile to optimize (reduce) times to as low as 10-15 seconds for both denaturation and annealing steps, which will greatly reduce overall PCR time.

GC-rich targets

If having troubles amplifying GC-rich targets, one could try adding 5-10% DMSO, up to 10% glycerol, or 1-2% formamide (or any combination of these solvents) to the reaction mixture. Note that the optimal Ta will decrease approximately 5ºC in 10% DMSO.