# GRS Hotstart Taq DNA Mastermix

**#GE73.0100 | # GE73.5100**

(for research only)

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**Product:**

GRS Hotstart Taq DNA polymerase consists of GRS Taq bound to a specific antibody that blocks polymerase activity, preventing or minimizing the synthesis of non-specific products and primer-dimers prior to PCR cycling, which results in an overall higher specificity. The heat-labile antibody can be rapidly inactivated by increasing the temperature for a few minutes to 95ºC (hot-start) resulting in polymerase activation. Like GRS Taq, this enzyme has 5´→3´ polymerase activity but no 3´→5´ exonuclease activity (no proofreading). It is suitable for amplifying DNA targets up to 5kb and produces 3´ A-overhangs allowing TA-cloning

**Source:**

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

**Quantity:**

GRS Hotstart Taq DNA Polymerase is supplied as a convenient 2x mastermix containing all required components, except specific primers. One vial of 1,25 ml is sufficient for 100 reactions of 25µl (or 125 reactions of 20µl). Final concentration of $MgCl_2$ will be 3mM.

**Contents:**

| Product | GE73.0100 | GE73.5100 |
|---|---|---|
| GRS Hotstart Taq Mastermix (2x) | 1,25ml | 5x 1,25ml |

**Applications:**

Routine PCR

**Properties:**

Amplicon size: up to 5kb.
Extension Rate 1kb/min
Hotstart: Yes
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 Hotstart Taq-concentration, and need to be determined case by case. We suggest to start with the following basic protocol and subsequently optimize annealing temperature, incubation times and cycling number.

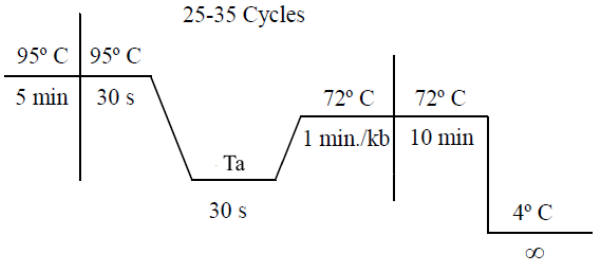
Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with GRS HotstartTaq:

| Component | Volume | Final Conc. |
|---|---|---|
| GRS Hotstart Taq Mastermix (2X) | 12,5 µl | 1X |
| Forward primer (5 pmol/ µl) | 1,5 µl | 0,3 µM |
| Reverse primer (5 pmol/ µl) | 1,5 µl | 0,3 µM |
| Template DNA | 0,25 - 10 µl | 2-200 ng/µl* |
| PCR –grade water | up to 25 µl | |

*) In case of plasmid DNA use 1-3 ng, in case of gDNA add 200-300ng
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 in 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 (antibody inactivation and 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 ligate (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 Hotstart Taq Mastermix contains 6mM $MgCl_2$ (final concentration is 3mM). If desired one could optimize the final $MgCl_2$ –concentration further in steps of 0.25mM by adding the required amount of 25mM $MgCl_2$ (not provided)**.**

**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 10-20 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.