

TAQKLE Hotstart Mastermix with Dye (2x)

#GE81.0001 (1.25ml) | GE81.5001 (5x 1.25ml) | GE81s (trial size)
 (FOR RESEARCH ONLY)



Product: TAQKLE Hotstart DNA Polymerase is the to go to enzyme for challenging DNA samples, including targets with high GC content (up to 80%), sequences with secondary structures, long targets and samples with presence of inhibitors. Its enhanced DNA binding capacity results in improved processivity, shorter extension times, higher yield and the capacity to tackle more difficult and longer targets.

Hotstart is achieved using a proprietary aptamer-like molecule that reversibly inhibits both 5'-3' polymerase activity and 3'-5' exonuclease activity. It prevents the formation of primer-dimers and non-specific amplification, increasing the sensitivity and specificity of the PCR reaction.

Moreover, this allows preparing reaction mixtures at room temperature with pre- and post-PCR stability for up to 24h. DNA binding has been improved by protein engineering resulting in shorter extension times (2-6kb/min), making this not only a real problem-solving enzyme for difficult targets but also allows for very fast PCR amplifications.

For convenience an inert red dye has been added to the mastermix as a visual pipetting aid and it also allows for direct loading onto an agarose gel.

Applications: GC/AT-rich targets, presence of inhibitors, DNA with secondary structures, long targets

Contents: TAQKLE Hotstart is supplied as a 2X Mastermix consisting of optimized concentrations of MgCl₂, dNTPs, enhancers and stabilizers.

Component	GE81.0001	GE81.5001	GE81s
TAQKLE Hotstart Mastermix with Dye (2X)	1.25ml	5x 1.25ml	0.5ml

QC: Functionally tested in PCR. Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays

Properties:
 Extension rate: 2-6kb/min
 Hotstart: Yes (reversible)
 A-overhang: No

Storage: -20°C and protected from light for at least 1 year. No loss of performance is detected after 20 freeze/thaw cycles.

Prior to use

Optimal PCR cycling conditions (incubation times, temperatures, and concentrations) depend on DNA target (GC-content, size, quantity, purity, etc.), specific primers, dNTPs, buffer composition and MgCl₂-concentration, and need to be determined case by case. TAQKLE Hotstart MasterMix (2x) already includes dNTPs and has been already been optimized with respect to the MgCl₂ concentration and other components to maximize success rates. Thus, it is not recommended to add additional MgCl₂ or dNTPs. We suggest starting with the following basic protocol and subsequently optimizing annealing temperature, incubation times and cycling number

Basic Protocol

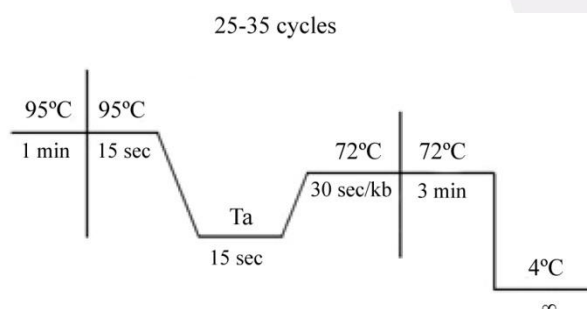
1. Mix for each PCR reaction:

Component	Volume (25µl)	Final Conc.
TAQKLE Hotstart Mastermix (2X)	12.5 µl	1X
Forward primer (5 pmol/µl)	2 µl	0.4 µM
Reverse primer (5 pmol/µl)	2 µl	0.4 µM
Template DNA*	0.25 – 10 µl	1-100 ng*
PCR-grade water	up to 25 µl	

*) In case of gDNA <100ng (total amount) and in case of complex DNA (e.g., GC-rich) <5ng
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.

2. Set-up initial PCR amplification as follows:



After an initial cycle of 1 min at 95°C (denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 25-35 times for 15 seconds at 95°C, 15 seconds at Ta, and 30 seconds per kb of target DNA at 72°C 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 3 min at 72°C. Analyze PCR products by DNA Agarose gel electrophoresis. Samples can be loaded directly onto an agarose gel without the need of adding a loading buffer. Using a 1% agarose gel, the inert red tracking dye co-migrates with DNA of approximately 600bp and using a 2% with DNA of approximately 350bp.

Optimization

Denaturation

If amplification of GC-rich templates results in low yield, increasing temperature to 98°C-100°C may improve amount of product.

Annealing Temperature (Ta) and Primers

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 (e.g., starting at the lowest Tm and increasing with 2°C increments). Ideally, primers have melting temperatures of approximately 60°C and final concentration should be between 0.2 and 0.6µM (each).

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 seconds for both denaturation and annealing steps, which will greatly reduce overall PCR time. Optimal extension is carried out with a velocity of 2kb/min but may be reduced to as little as 6kb/min for less demanding templates.