Xpert AmpliFi Hotstart

(dNTPs included)

#GE37.0100 (100 U) | GE37s (trial size)

(FOR RESEARCH ONLY)

Product: Xpert AmpliFi Hotstart DNA Polymerase is an all-round and robust high-fidelity enzyme with enhanced DNA binding, resulting in improved processivity, shorter extension times, higher yield and capacity to amplify more difficult and longer targets. Combined with its extremely low error-rate and all the advantages of a new proprietary reversible hotstart technology, this makes Xpert AmpliFi Hotstart ideal for applications such as high-fidelity PCR, site-directed mutagenesis, multiplexing, long-range PCR and NGS sequencing, when robustness, specificity, sensitivity and proof-reading are extremely important.

Xpert AmpliFi Hotstart DNA polymerase has been derived from Pfu DNA polymerase for its proof-reading activity. Xpert AmpliFi has a very high accuracy resulting in fidelity that is about 100x higher than regular Taq DNA polymerase. 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 also an ideal choice for consistent results in fast PCR amplifications.

Applications: High-Fidelity PCR, cloning, site-directed mutagenesis. Long-Range PCR, Multiplex PCR, NGS sequencing

Contents: Xpert AmpliFi Hotstart is supplied with 1 vial of 1.7ml of Reaction Buffer (5x) consisting of optimized concentrations of $MgCl_2$, dNTPs, enhancers and stabilizers, and 1 vial of 1.7ml Enhancer Mix for difficult and/or long targets (e.g., GC-rich or with complex secondary structures). #GE37s is a trial sample.

Component	GE37.0100	GE37s
Xpert AmpliFi Hotstart (2U/µl)	50µl	10µl
Amplifi HS Reaction Buffer (5x)	1.7ml	200µl
Amplifi HS Enhancer Mix (10x)	1.7ml	300µl

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

Properties:

Amplicon size:	up to 20kb
Extension rate:	2-6kb/min
Hotstart:	Yes (reversible)
A-overhang:	No
Error-rate:	100x lower than normal Taq

Storage: -20ºC 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 $MgCl_2$-concentration, and need to be determined case by case. AmpliFi HS Reaction Buffer already includes dNTPs and has been already been optimized with respect to the $MgCl_2$ concentration and other components to maximize success rates. Thus, it is not recommended to add additional $MgCl_2$ or dNTPs. We suggest starting with the following basic protocol and subsequently optimizing annealing temperature, incubation times and cycling number. In case no amplification is observed, it is recommended to add AmpliFi HS Enhancer Mix as it may improve performance, especially for some difficult and/or long templates.

Basic Protocol

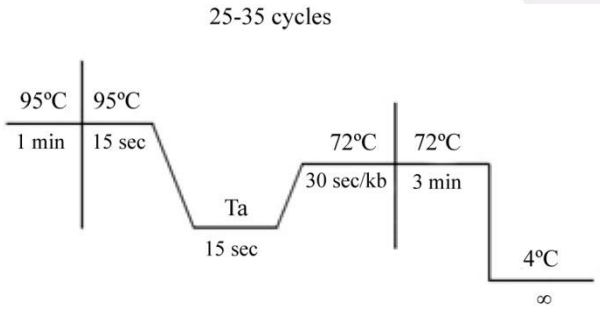
1. Mix for each PCR reaction:

Component	Volume (25µl)	Final Conc.
AmpliFi HS Reaction Buffer (5x)	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*
Xpert AmpliFi Hotstart (2U/µl)	0.25 µl	0.05 U/µl
AmpliFi Enhancer Mix (10x) [**optional**]	2.5 µl	1X
PCR–grade water	up to 25 µl	

*) *In case of* gDNA <100ng (total amount) and in case of complex DNA using AmpliFi Enhancer <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.

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.