KFX HiFi 2X PCR Master Mix with HF Buffer

WEB: www.abclonal.com

Catalog: RK20692
Size: 100 RXN / 500 RXN
Concentration: 2 X
Components:

KFX HiFi 2X PCR Master Mix with HF Buffer	RM20361

Product Description

ABclonal KFX HiFi DNA Polymerase is a novel B-family DNA polymerase engineered to have increased affinity for DNA. The intrinsic high processivity of the enzyme results in significant improvement in yield, speed and sensitivity when compared with wild-type B-family DNA polymerases. In addition, the ability to amplify long targets, as well as GC- and AT-rich targets, is significantly improved.

KFX HiFi DNA Polymerase has 5'→3' polymerase and 3'→5' exonuclease (proofreading) activity, but no 5'→3' exonuclease activity. The strong 3'→5' exonuclease activity results in superior accuracy during DNA amplification, lending to KFX HiFi DNA Polymerase the lowest published error rate of all B-family DNA polymerases (1 error per 3.6 x 10^6 nucleotides incorporated). This fidelity is approximately 100 times higher than that of wild-type *Taq* DNA polymerase, and up to 10 times higher than that of other B-family DNA polymerases and polymerase blends.

In KFX HiFi 2X PCR Master Mix with HF Buffer, ABclonal KFX HiFi DNA Polymerase is supplied in a convenient 2X Master Mix format, containing all reaction components except primers and template. The Master Mix contains ABclonal KFX HiFi DNA Polymerase (1 U per 50 µL reaction) in a proprietary reaction buffer containing dNTPs (0.3 mM of each dNTP at 1X), $MgCl_2$ (1.5 mM at 1X) and stabilizers. KFX HiFi 2X PCR Master Mix with HF Buffer is designed for routine, high-fidelity PCR of a wide range of targets and fragment sizes. In addition, ABclonal KFX HiFi DNA polymerase requires significantly shorter reaction times than wild-type B-family DNA polymerases.

DNA fragments generated with KFX HiFi 2X PCR Master Mix may be used for routine downstream analysis and applications, including restriction enzyme digestion, cloning and sequencing. PCR products generated with KFX HiFi 2X PCR Master Mix are blunt-ended but may be 3'-dA-tailed for cloning into TA cloning vectors.

Storage Temperature: -20 ℃
Heat Inactivation: No
5' - 3' Exonuclease: No
3' - 5' Exonuclease: Yes

Note:

- KFX HiFi 2X PCR Master Mix contains the engineered ABclonal KFX HiFi DNA Polymerase – developed for fast and versatile high-fidelity PCR.
- Buffer, $MgCl_2$, dNTPs and enzyme are supplied in a convenient 2X Master Mix – just add primers and template.
- ABclonal KFX HiFi DNA Polymerase has the lowest published error rate of all B-family DNA polymerases (1 error per 3.6 x 10^6 nucleotides incorporated).
- Amplify targets up to 15 kb from genomic DNA or 20 kb from less complex targets.
- Denature at 98 ℃ for 20 sec per cycle.
- Optimal annealing temperatures are typically higher than in other PCR buffer systems. Use an annealing temperature gradient to determine the optimal annealing temperature.
- To ensure the highest fidelity, use high quality DNA and the lowest possible number of cycles.
- KFX HiFi 2X PCR Master Mix with HF Buffer (RK20692) contains KFX HF Buffer. The error rate of KFX HiFi DNA Polymerase in HF Buffer is even lower than that in GC Buffer; therefore, the Master Mix with HF Buffer should be used as a default for high-fidelity amplification. However, GC Buffer can improve the performance of KFX HiFi DNA Polymerase on some difficult or long templates, i.e. GC-rich templates or those with complex secondary structures. ABclonal KFX HiFi 2X PCR Master Mix with GC Buffer (RK20696) offers robust performance on some difficult or long templates. KFX HiFi 2X PCR Master Mix for NGS (RK20698) is optimized for amplification of NGS libraries. This formulation is designed for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries, and further improves the uniformity of amplification of libraries, including superior performance with GC-rich regions.

Name	Catalog	Application
KFX HiFi 2X PCR Master Mix with HF Buffer	RK20692	Routine, high-fidelity PCR of a wide range of targets and fragment sizes
KFX HiFi 2X PCR Master Mix with GC Buffer	RK20696	PCR on difficult or long templates, i.e. GC-rich templates or those with complex secondary structures
KFX HiFi 2X PCR Master Mix for NGS	RK20698	Robust, high-fidelity amplification of NGS libraries

Product Applications

KFX HiFi 2X PCR Master Mix with HF Buffer is ideally suited for:

- PCR for conventional sequencing (direct sequencing or sequencing of cloned PCR products).
- Next-generation sequencing library amplification.
- Amplification of DNA fragments for cloning and protein expression or genomic characterization.
- Site-directed mutagenesis.

Standard PCR Protocol

IMPORTANT! The KFX HiFi Master Mix contains an engineered B-family (proofreading) DNA polymerase and uniquely-formulated buffers and requires specialized reaction conditions. If these conditions are not adhered to, reaction failure is likely. Refer to Important Parameters for more information.

Step 1: Prepare the PCR master mix

- KFX HiFi 2X PCR Master Mix reactions MUST be set up on ice since the high proofreading activity of the enzyme will result in rapid primer degradation at room temperature.
- Ensure that all reagents are properly thawed and mixed.
- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.
- Calculate the required volumes of each component based on the following table:

Component	25 µL Reaction[1]	50 µL Reaction[1]	Final conc.
PCR-grade water	Up to 25 µL	Up to 50 µL	N/A
KFX HiFi 2X PCR Master Mix[2,3]	12.5 µL	25 µL	1X
10 µM Forward Primer	0.75 µL	1.5 µL	0.3 µM
10 µM Reverse Primer	0.75 µL	1.5 µL	0.3 µM
Template DNA[4]	As required	As required	As required

1. Reaction volumes may be adjusted between 10–50 µL. Reaction volumes >50 µL are not recommended.

2. KFX HiFi PCR Master Mix contains 1.5 mM $MgCl_2$ (1X). Additional $MgCl_2$ may be added separately but is unlikely to be required.

3. KFX HiFi PCR Master Mix contains 0.3 mM of each dNTP (1X), and 1 U of ABclonal HiFi DNA Polymerase (per 50 µL reaction) in a proprietary reaction buffer.

4. Use <100 ng genomic DNA (10–100 ng) and <1 ng less complex DNA (0.1–1 ng) per 25 µL reaction as first approach.

Step 2: Set up individual reactions

- Transfer the appropriate volumes of PCR master mix template and primer to individual PCR tubes or wells of a PCR plate.
- Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation[1]	95 ℃	3 min	1
Denaturation[2]	98 ℃	20 sec	15–35[6]
Annealing[3,4]	60–75 ℃	15 sec	
Extension[5]	72 ℃	15–60 sec/kb	
Final extension	72 ℃	1 min/kb	1

1. Initial denaturation for 3 min at 95 ℃ is sufficient for most applications. Use 5 min at 95 ℃ for GC-rich targets (>70% GC content).
2. KFX HiFi PCR Master Mix has a higher salt concentration than conventional PCR ready-mixes, which affects DNA melting. To ensure that complex and GC rich targets are completely denatured, use a temperature of 98 ℃ for denaturation during cycling.
3. In addition to DNA melting, high salt also affects primer annealing. The optimal annealing temperature for a specific primer set is likely to be different (higher) than when used in a conventional PCR ready-mix. An annealing temperature gradient PCR is recommended to determine the optimal annealing temperature with the KFX HiFi PCR Master Mix. If gradient PCR is not feasible, anneal at 65 ℃ as a first approach.
4. Two-step cycling protocols with a combined annealing/extension temperature in the range of 68–75 ℃ and a combined annealing/extension time of 30 sec/kb may be used.
5. Use 15 sec extension per cycle for targets ≤1 kb, and 30–60 sec/kb for longer fragments, or to improve yields.
6. For highest fidelity, use ≤25 cycles. In cases where very low template concentrations or low reaction efficiency results in low yields, 30–35 cycles may be performed to produce sufficient product for downstream applications.