

TECHNICAL DATA SHEET

DeCodi-Fi™

High-Fidelity PCR Kit

Product Code: DeCodiFi_HFPK-100 rxn

DeCodiFi_HFPK-400 rxn

PRODUCT DESCRIPTION

DeCodi-Fi™ High-Fidelity PCR Kit features a recombinant Hotstart High Fidelity Polymerase designed for exceptional processivity and potent proofreading capabilities. It has low amplification bias and provides consistent sequencing coverage, delivering more reliable results.

Tailored for routine high-fidelity PCR, the DeCodi-Fi™ High-Fidelity PCR Kit empowers you with precise and robust amplification. It minimizes non-specific product formation while maximizing target yield, even when dealing with minute input quantities as low as 1 ng. Additionally, this kit includes two buffers: a 5X High-Fidelity buffer recommended for amplifying most templates which have balanced GC/AT content, and a 5X GC-rich Buffer recommended for amplifying GC-rich targets. Whether you're conducting routine PCR or tackling GC-rich amplifications, our DeCodi-Fi™ High-Fidelity PCR Kit provides the tools you need for success.

PRODUCT APPLICATIONS

- Amplification of DNA fragments for cloning
- Long-range PCR and GC rich templates
- Library amplification for sequencing

SHIPPING AND STORAGE

The DeCodi-Fi™ High-Fidelity PCR kit is designed to be transported at 2–8 °C without loss of performance for up to 7 days. This reduces environmental impact by minimizing thermal packaging, eliminating dry ice, and simplifying logistics.

Storage recommendations:

- Store at –20 °C upon arrival.
- Avoid repeated freeze–thaw cycles.
- Do not use damaged or expired components. If damage occurs during shipment, contact Kura Biotech technical support at sales@blikka.com

Components	Volume 100 rxn kit	Volume 400 rxn kit	Storage T°
DeCodi-Fi™ High-Fidelity Polymerase	50 µL	200 µL	–20°C
DeCodi-Fi™ 5X High-Fidelity Buffer	500 µL	1.75 mL	
DeCodi-Fi™ 5X GC-rich Buffer	500 µL	1.75 mL	
DeCodi-Fi™ MgCl ₂ (25 mM)	400 µL	800 µL	
DeCodi-Fi™ dNTP mix (10 mM)	150 µL	300 µL	

STANDARD PCR RECIPE

Calculate the volume of reagents needed for each reaction. Typically, reactions are set up in 25 or 50 µL volumes.

General recipe for a 25 µL reaction:

Components	25 µL reaction	Final concentration
Nuclease-free water	To 25 µL	-
DNA template	^a	1 ng–100 ng ^c
Forward primer (10 µM)	0.5 µL	0.2 µM
Reverse primer (10 µM)	0.5 µL	0.2 µM
DeCodi-Fi™ 5X High-Fidelity Buffer or 5X GC-rich Buffer	5 µL	1X
DeCodi-Fi™ MgCl ₂ (25 mM)	^b	^b
DeCodi-Fi™ dNTP Mix (10 mM)	0.75 µL	0.3 mM
DeCodi-Fi™ High-Fidelity Polymerase (1U/µL)	0.5 µL	0.02 U/µL

^aThe volume used depends on the concentration of the template and the desired input amount.

^bThe buffer contains 2 mM Mg²⁺ at 1X concentration. Additional MgCl₂ can be added if necessary.

^c **Recommended DNA Template:**

- 5-10 ng for prokaryotic genomes.
- 10–50 ng for eukaryotic genomes.
- ≤1 ng for plasmids, phages or templates ≤ 50 Kb.

Always use more than 10⁴ DNA template copies for optimal amplification (see approximate DNA mass equivalents for different sources in the table below)

DNA source	Nanograms (for >10 ⁴ copies)
Bacteriophage λ (lambda)	>0.0005 ng
<i>Escherichia coli</i>	>0.05 ng
<i>Arabidopsis thaliana</i>	>1.45 ng
Human Genomic DNA	>34 ng

Primer Design:

It is recommended to incorporate two **phosphorothioate bonds** at the 3'-ends of primers to prevent 3'-exonuclease degradation (proofreading), enhance specificity and avoid adapter dimer formation.

PREPARE THE PCR REACTION

- To prevent primer degradation caused by DeCodi-Fi™'s strong 3'-exonuclease activity, set up the PCR reaction on ice.
- Mix all components in a sterile PCR tube or plate and centrifuge.
- Place the PCR tubes or plates into the thermal cycler.
- Set up the cycling conditions based on the primer's calculated melting temperature (T_m)^e or according to results from a previous gradient PCR (strongly recommended).
It is recommended to use the lower primer T_m as a reference when performing a temperature gradient. Start 4 °C below this value and increase in 2 °C increments up to 4 °C above the calculated T_m.

PCR CYCLING PROGRAM

Step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1
Denaturation	95°C ^d	5-10 sec	10-35 ^f
Annealing	Calculated T _m ^e	15 sec	
Extension	72°C	15 sec/kb	
Final extension	72°C	2 min	1
Hold	4°C	∞	-

^d Use 98°C for 10 sec for GC-rich templates (>70% GC).

^e Suggested T_m calculated with default parameters and "salt adjusted" using:

[Oligonucleotide Properties Calculator](#)

If using GC buffer we suggest starting with an annealing temperature of 3°C below the calculated T_m.

^f Cycle numbers may need to be optimized based on specific template input, primers, and final application. Lower cycling reduces the probability of errors, and helps diminishing nonspecific products or smearing.