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 has been designed by Kura Biotech to be transported within a temperature range of 2°C to 8°C without losing performance. Tests conducted have confirmed that these components can be kept under these conditions during transport for at least 7 days. Implementing these conditions in transportation helps reduce our carbon footprint by minimizing the use of thermal insulation, eliminating the need for dry ice, and simplifying logistics.

To ensure optimal kit performance, please adhere to the following guidelines:

- Store the components according to the specifications on each vial's label or follow the instructions in this manual. Avoid repeated freeze-thaw cycles.
- If any kit components were damaged during transportation, contact Kura Biotech. Do not use damaged or expired components as it may compromise performance.

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 MgCl2 (25 mM)	400 µL	800 µL	
DeCodi-Fi dNTP mix (10 mM)	150 µL	300 µL	

Standard PCR Protocol

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
dH_2O	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

[a] The volume used depends on the concentration of the template and the desired input amount.

[b] The buffer contains 2 mM Mg^{2+} at 1X concentration. Additional $MgCl_2$ can be added if necessary.

[c] Recommended input amounts are 5 - 50 ng for gDNA, and ≤1 ng for templates ≤ 50Kb. Load >10^4 copies of template for obtaining amplification at 25 cycles, while avoiding to use more than 100 ng per reaction (eg. 35 ng of human gDNA corresponds to 10^4 copies).

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 except the DeCodi-Fi High-Fidelity Polymerase in a sterile PCR tube or Plate.
- Add the DeCodi-Fi High-Fidelity Polymerase last and mix gently by pipetting up and down.

Perform the PCR Reaction:

- Place the PCR tubes or plates into the thermal cycler.
- Set up the cycling conditions based on the primer's calculated melting temperature or a previous gradient PCR.

A common PCR program consists of:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1
Denaturation	95°C[d]	5-10 sec	10-35[f]
Annealing	Calculated Tm[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 Tm 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 Tm.

[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.

PCR Product Analysis:

After PCR, you can analyze the products using gel electrophoresis, or you may proceed directly to downstream applications, depending on your experimental goals.

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Jun 10, 2025 v4.4