TECHNICAL DATA SHEET

DeCodi-Fi

2X All-in-One Mix

Product Code: DecodiFi-AIOM-1.25ml
DecodiFi-AIOM-5ml

PRODUCT DESCRIPTION

DeCodi-Fi 2X All-in-One Mix 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 or NGS library amplification, the DeCodi-Fi 2X All-in-One Mix 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, the All-in-One format provides our Hotstart High Fidelity Polymerase in a user-friendly 2X MasterMix configuration. This format includes all the necessary components for the reaction (dNTPs, $MgCl_2$ and stabilizers), excluding primers and template, in a proprietary buffer. Whether you're conducting routine PCR or NGS library amplification, our DeCodi-Fi 2X All-in-One Mix 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 2X All-in-One Mix 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 2X All-in-One Mix	1.25 mL	5 mL	-20°C

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 [b]
Forward primer (10 µM)	0.5 µL	0.2 µM
Reverse primer (10 µM)	0.5 µL	0.2 µM
DeCodi-Fi 2X All-in-One Mix	12.5 µL	1x

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

[b] 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 in a sterile PCR tube or plate and centrifuge.

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	98°C	5-10 sec[e]	10-35 [c]
Annealing	Calculated Tm[g]	15 sec	
Extension	68°C[f]	30 sec[d] + 15 sec/kb	
Final extension	68°C	2 min	1
Hold	4°C	∞	

[c] 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.

[d] Use 30 sec extension for amplicons < 1Kb, plus 15 sec/kb for longer fragments. If amplifying from a plasmid, extension times >30 sec may result in circular plasmid amplification, seen as a high molecular weight band in gels. Linearize the plasmid or lower the extension time.

[e] Use 98°C for 10 sec for GC-rich templates (>70% GC).

[f] 68°C is preferred for avoiding depurination. 72°C can be used for amplicons <7Kb.

[g] Suggested Tm calculated with default parameters and "salt adjusted" using:
Oligo Calc: Oligonucleotide Properties Calculator
http://biotools.nubic.northwestern.edu/OligoCalc.html

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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