TECHNICAL DATA SHEET

DeCodi-Fi™ Long&Complex

2X All-in-One Mix

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

PRODUCT DESCRIPTION

DeCodi-Fi™ Long&Complex 2X All-In-One Mix is a ready-to-use formulation containing the DeCodi-Fi™ Hot-Start High-Fidelity Polymerase optimized for challenging templates. It enables efficient amplification of long human DNA fragments (up to 30 kb), high-GC regions (up to 90% GC), and combined long/GC-rich targets (up to 20 kb with 70% GC). The mix efficiently amplifies low DNA inputs and templates containing expansion repeats.

The 2X All-In-One Mix includes DeCodi-Fi™ Hot-Start High-Fidelity Polymerase, dNTPs, $MgCl_2$, stabilizers, and GC enhancers in a proprietary buffer. Primers and template DNA are not included.

PRODUCT APPLICATIONS

- PCR Amplification of high-GC or complex DNA regions, including regulatory elements and repetitive sequences.
- Library amplification for long-read sequencers, including repetitive or high-GC/long targets requiring high-fidelity and robust performance.

SHIPPING AND STORAGE

The DeCodi-Fi™ Long&Complex 2X All-in-One Mix 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 (°C)
DeCodi-Fi™ Long&Complex 2X All-in-One Mix	1.25 mL	5 mL	-20°C

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	variable	≤ 100 ng [a] / 25 µL
Forward primer (10 µM)	0.5 µL	0.2 µM
Reverse primer (10 µM)	0.5 µL	0.2 µM
DeCodi-Fi™ Long&Complex 2X All-in-One Mix	12.5 µL	1x

[a] **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 10^4 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
Escherichia coli	>0.05 ng
Arabidopsis thaliana	>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 primer 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 (Tm)[b] or according to results from a previous gradient PCR (strongly recommended).
  To perform a temperature gradient, use the lower primer Tm calculated as reference. Start 4 °C below this value and increase in 2 °C increments up to 4 °C above the calculated Tm.

PCR CYCLING PROGRAM

Step	Temperature	Time	Cycles
Initial denaturation	98°C	1 min	1
Denaturation	98°C	10 sec	10-30 [c]
Annealing	Variable[b]	15 sec	
Extension	72°C	30 sec/kb	
Final extension	72°C	3 min	1
Hold	4°C	∞	

[b] Suggested Tm calculated with default parameters and "salt adjusted" using:
Oligonucleotide Properties Calculator

[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.

STEPDOWN PCR PROGRAM

For Expanded Repeats[d] or Non-specific Amplification in Long Amplicons

Phase	Step	Temperature	Time	Cycles
Initial Setup	Initial denaturation	94°C	2 min	1x
Phase 1	Denaturation	98°C	10 sec	5x
	Annealing/ Extension	74°C	30 sec/kb	
Phase 2	Denaturation	98°C	10 sec	5x
	Annealing/ Extension	72°C	30 sec/kb	
Phase 3	Denaturation	98°C	10 sec	6x
	Annealing/ Extension	70°C	30 sec/kb	
Phase 4	Denaturation	98°C	10 sec	15x
	Annealing/ Extension	68°C	30 sec/kb	

[d] For **expanded repeats** with 100% GC-rich content, please contact our technical support team at sales@blikka.com. They can provide guidance on the most appropriate solution for your specific application.

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Nov 17, 2025 v1