TECHNICAL DATA SHEET

DeCodiFi™ Alpha

2X All-in-One Mix

Product Code: DCA-5ml, DCA-1.25ml

PRODUCT DESCRIPTION

DeCodiFi™ Alpha 2X All-in-One Mix features a recombinant Hotstart High-Fidelity Polymerase engineered to deliver exceptional accuracy, high amplification yield, and uniform coverage across sequencing libraries. Its low amplification bias ensures consistent sequencing coverage, delivering highly reliable results.

Tailored for high-performance NGS library amplification and demanding high-fidelity PCR applications, this mix provides robust and precise amplification with improved library uniformity. It maximizes target yield while preserving sequence integrity, even from low-input amounts down to 100 pg.

The convenient All-in-One 2X Master Mix format includes our engineered hot-start High-Fidelity Polymerase, dNTPs, MgCl2, and proprietary stabilizers in an optimized buffer system, requiring only the addition of primers and template.

PRODUCT APPLICATIONS

- Amplification of DNA fragments
- Library amplification for sequencing

SHIPPING AND STORAGE

The DeCodiFi™ Alpha 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°
DeCodiFi™ Alpha 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	[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
DeCodiFi™ Alpha 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 DNA Template**:

- 5-10 ng for prokaryotic genomes.
- 10–50 ng for eukaryotic genomes.
- ≤1 ng for plasmids, phages or templates sizes ≤ 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 adapter dimer formation.

PREPARE THE PCR REACTION

⚠️ **IMPORTANT: ALWAYS WORK ON ICE**

To prevent primer degradation caused by DeCodiFi™'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)[d] 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.

Note: Always prepare fresh dilutions under 2 ng/ml and eliminate after use.

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

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

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

[e] 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.

E.Coli genomic library template (ng)	Cycles
0.5	14-15
1	13-14
2.5	12-14
5	11-12
10	10-11
25	8-9

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