TECHNICAL DATA SHEET

DeCodi-Fi™ Plant Direct

2X All-in-One Mix

Product Code: PD-AIOM-DeCodiFi-1.25ml, PD-AIOM-DeCodiFi-5ml

PRODUCT DESCRIPTION

DeCodi-Fi™ Plant Direct 2X All-In-One Mix is a ready-to-use mix featuring DeCodi-Fi™ Hot-Start High-Fidelity Polymerase optimized to tolerate inhibitors from a wide range of plant species and tissues. It enables direct amplification from crude extracts, eliminating the need for DNA purification. The optimized formulation delivers high yield, accuracy, and reliability, even with challenging plant types. Compatible with multiplex PCR. The mix includes Hot-Start Polymerase, dNTPs, magnesium, enhancers, and proprietary stabilizers. Primers and template DNA are not included. Ideal for plant genotyping, transgene detection, and routine PCR directly from plant material.

PRODUCT APPLICATIONS

- Direct PCR
- Genotyping by sequencing
- Transgene detection

SHIPPING AND STORAGE

The DeCodi-Fi™ Plant Direct 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°
DeCodi-Fi™ Plant Direct 2X All-in-One Mix	1.25 mL	5 mL	-20°C

RECOMMENDED LYSIS PROTOCOL

- Cut 1–4 mm² of plant tissue and place it in 50 µL Buffer A (25 mM NaOH, 0.2 mM EDTA).
- Incubate at 95 °C for 1 hour, then cool to room temperature.
- Add 50 µL Buffer B (40 mM Tris-HCl, final pH 8.7–9.0) to neutralize and mix gently.
- Keep the lysate on ice (4 °C) if used immediately. For longer storage, keep at −20 °C. Avoid multiple freeze–thaw cycles.
- For PCR, use the supernatant (plant crude extract) as the DNA source. Add up to 20% of the total reaction volume (e.g., 5 µL in a 25 µL reaction).

Please note: Buffer A and Buffer B are not included in the kit and must be prepared by the user.

General recipe for a 25 µL reaction:

Components	25 µL reaction	Final concentration
Nuclease Free Water	To 25 µL	-
Plant crude extract[a]	1.25 µL -5 µL	**5-20%**
Forward primer (10 µM)	0.5 µL	0.2 µM
Reverse primer (10 µM)	0.5 µL	0.2 µM
DeCodi-Fi Plant Direct 2X All-in-One Mix	12.5 µL	1x

[a] Most plant species perform well using 20% of the HotShot extract in the PCR reaction. For plants with high levels of inhibitors, such as strawberry leaves, it is recommended to dilute the HotShot extract 1:4 and continue using 20% of the diluted extract in the reaction.

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 6 °C below this value and increase in 2 °C increments up to 6 °C above the calculated Tm.

PCR CYCLING PROGRAM

Step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1
Denaturation	95°C[c]	10 sec	30-40 [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.

MULTIPLEX PCR GUIDELINES

- **Primer validation:** Test each primer pair individually under standard conditions before multiplexing to confirm specificity and product size.
- **Primer design:** Use primers of 20–28 nt in length, 40–60% GC content (preferably 50–60%), and similar Tm values (≥60°C). Avoid 3' complementarity, long G/C runs, and secondary structures.
- **Primer concentration:** Start with 0.05 µM of each primer. If yield is low, increase gradually up to 0.2 µM.
- **Reaction setup:** Use standard cycling parameters and adjust extension time according to the longest fragment in the mix. Reactions can be scaled up to 50 µL to accommodate more primer pairs.
- **Annealing temperature**: Set according to the lowest Tm among primer pairs. If non-specific bands appear, increase in 1.5°C increments; if bands are missing, lower in 1.5°C steps.
- **Cycle optimization:** Begin with 30–35 cycles; reducing cycle number can minimize non-specific amplification and smearing.

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