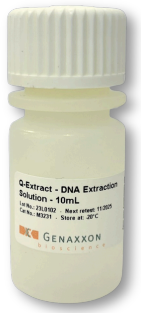
APPLICATON NOTE

Plant DNA Q-Extraction Solution combined with qPCR using InhibiCore Rapid Plant PCR MasterMix (5X)



PCR-ready DNA from plants

Plant DNA Q-Extraction Solution (M3230) is perfectly suitable for rapid and efficient extraction of PCR-ready DNA from plant leaves. The non-toxic Plant DNA Q-Extraction Solution enables the extraction of PCR-ready DNA in just 8 minutes. The PCR-ready DNA is ideal for end-point PCR. Here we describe how PCR-ready DNA extracted from wheat (*Triticum aestivum*) and tomato (*Solanum lycopersicum*) are used for successful realtime PCR using the Genaxxon InhibiCore Rapid Plant PCR MasterMix (5X) (M3250) with 1x GreenDye.

1) Sensitive qPCR analysis of chlorophyll gene targets from wheat lysates

We demonstrate the reliable and sensitive detection of chlorophyll gene targets from plant lysates using qPCR with fluorescent dye chemistry. The assay was evaluated using a serial dilution of wheat lysate (10%, 1%, 0.1%, 0.01%, 0.001%) alongside a no-template control (NTC) to assess sensitivity and inhibition tolerance across a broad range of sample concentrations.

qPCR reactions were performed under the following 2-step cycling conditions: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 sec and 60 °C for 30 sec. A subsequent melt curve analysis was included to verify amplification specificity. Fluorescence was detected in the FAM channel, corresponding to the fluorescent dye signal.

At the highest lysate concentration (10%), a reduction in fluorescence signal intensity was observed, indicating partial signal quenching. However, successful amplification was still achieved across all tested dilutions, demonstrating the robustness of the system against inhibitory compounds present in plant material (figure 1).

Melt curve analysis revealed a single, distinct peak for all positive samples, confirming the formation of a single, specific amplicon and absence of non-specific amplification or primer-dimer formation (figure 1).

Plant lysates were prepared using a rapid 8-minute extraction protocol as described in the manual for the Plant DNA Q-Extraction Solution (M3230), enabling fast and efficient sample preparation without the need for conventional DNA purification steps.

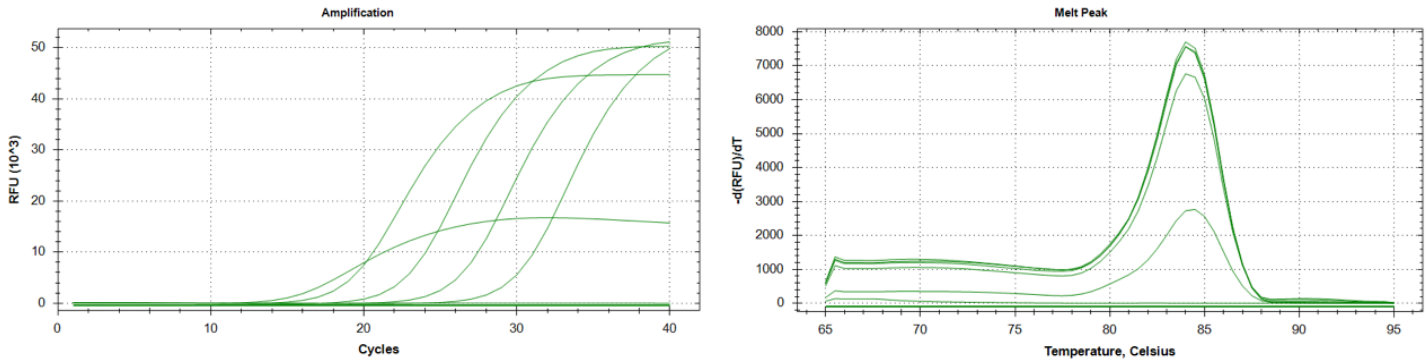


Figure 1. qPCR data by wheat lysate qPCR with InhibiCore Rapid Plant PCR MasterMix (5X)
The figure shows qPCR amplification curves and melting curve analysis of a wheat lysate dilution series (10% to 0.001%) and a no-template control (NTC) using InhibiCore Rapid Plant PCR MasterMix (5X) and 1xGreenDye. Clear, concentration-dependent amplification is observed in the FAM channel, with reduced signal intensity at 10% lysate due to matrix effects. Melting curve analysis reveals a single specific peak for all positive samples, confirming specific target amplification without non-specific products.

2) Benchmark of Direct PCR performance from 10% tomato lysate using InhibiCore Rapid Plant PCR MasterMix (5X) compared to competitor mixes

We compared the performance of InhibiCore Rapid Plant PCR MasterMix (5X) with three competitor master mixes for qPCR from 10% tomato lysate prepared using the Plant DNA Q-Extraction Solution (M3230) protocol. The evaluation was performed using qPCR with fluorescent dye detection in the FAM channel to assess amplification efficiency and inhibitor tolerance under challenging sample conditions.

qPCR reactions were carried out under the following cycling conditions: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 sec and 60 °C for 30 sec. A subsequent melt curve analysis was included to confirm amplification specificity.

InhibiCore Rapid Plant PCR MasterMix (5X) and Competitor N both produced detectable amplification curves, while Competitor T and Competitor Q showed strong inhibition with no significant amplification signals observed. Among all tested systems, InhibiCore Rapid Plant PCR MasterMix (5X) demonstrated the earliest Ct value, indicating superior performance and the highest tolerance to PCR inhibitors present in crude tomato lysates (figure 2).

Melt curve analysis confirmed specific amplification in successful reactions, with single distinct peaks observed and no evidence of non-specific products or primer-dimer formation (figure 2).

Tomato lysates were prepared using a rapid 8-minute Plant DNA Q-Extraction Solution (M3230) protocol as described in the corresponding manual, enabling direct use in qPCR without conventional DNA purification steps.

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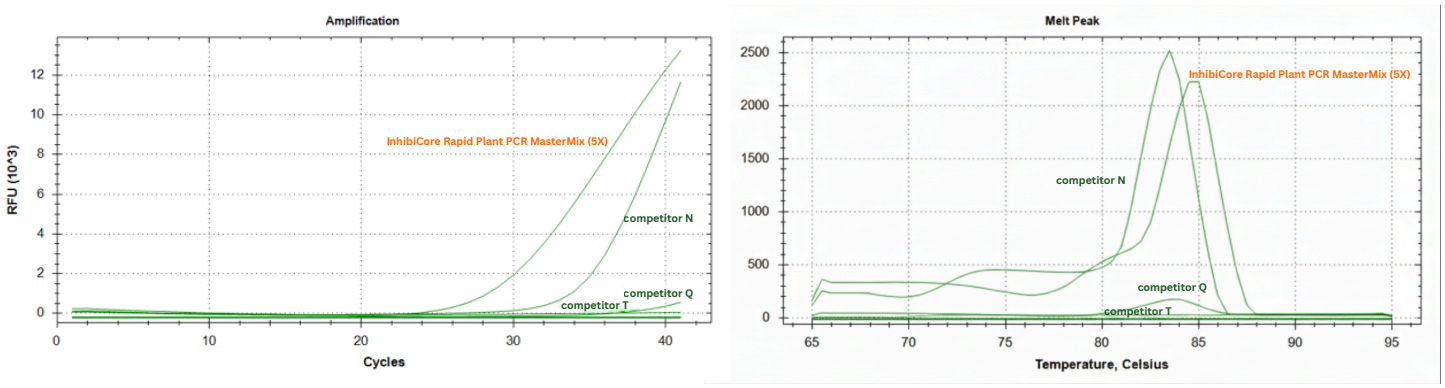
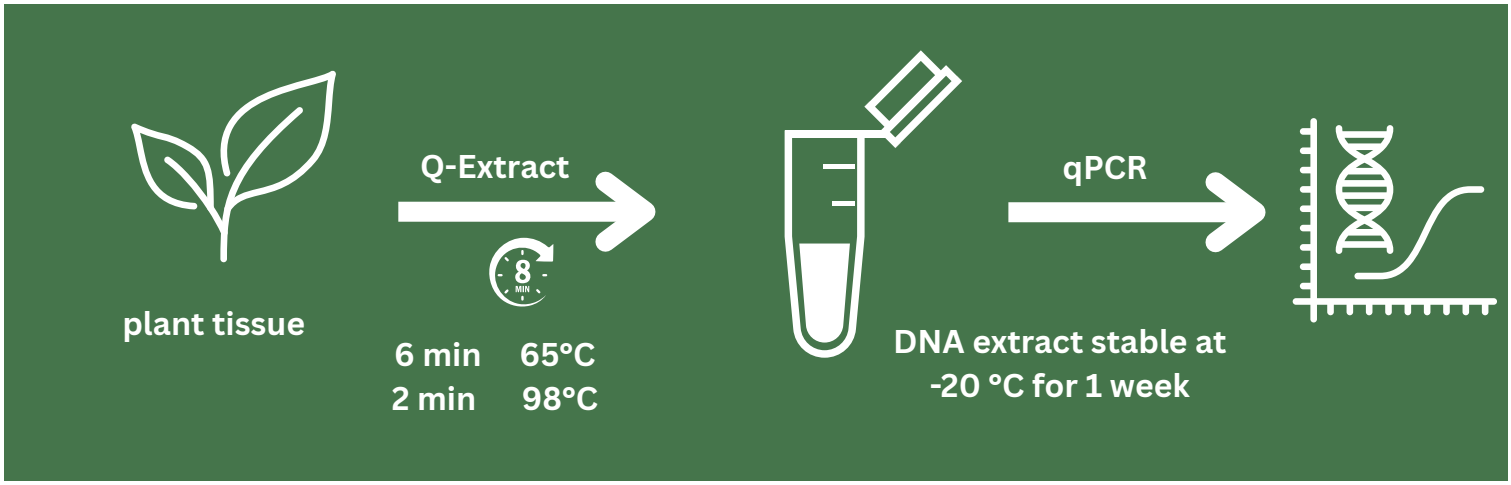


Figure 2. Plant direct PCR from plant samples Benchmark - inhibitor effect from Tomato lysate
Direct PCR was performed using a cycling protocol of 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 sec and 60 °C for 30 sec, with subsequent melt curve analysis. InhibiCore Rapid Plant PCR MasterMix (5X) and Competitor N showed successful amplification, while Competitor T and Competitor Q were strongly inhibited and did not generate reliable amplification curves. InhibiCore Rapid Plant PCR MasterMix (5X) exhibited the earliest Ct value among all tested systems, indicating the highest tolerance to plant-derived PCR inhibitors and superior performance in crude tomato lysates.

Conclusion:

Overall, the data demonstrate that InhibiCore Rapid Plant PCR MasterMix (5X) provides robust, sensitive, and inhibitor-tolerant performance for direct PCR applications from crude plant material, outperforming tested competitor systems.

Workflow



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Protocol

Extraction Protocol with Plant DNA Q-Extraction Solution M3230

Note: Preparation of DNA extraction should be performed in a separate area from that used for setting up the PCR reaction.

1. Thaw DNA Q-Extraction Solution.
For the first time use, aliquot the Q-Extract DNA Extraction solution into smaller volumes. (DNA Q-Extraction Solution has a cloudy appearance).

2. Add ca. 10mg of plant leaves to a tube containing 100µL Plant DNA Q-Extraction Solution.
Make sure that the leaves are completely covered by the Plant DNA Q-Extraction Solution.

3. Vortex the tube for 15 sec.

4. Transfer the tube to a heat block or a thermal cycler and incubate at

1. 65°C for 6 min
2. 98°C for 2 min
3. 4°C (or cool down on ice)

The DNA extract is now ready for qPCR.
DNA extracts are stable at -20°C for one week or long-term at -80°C.

PCR protocol for plant DNA extracted with Plant DNA Q-Extraction Solution M3230

PCR protocol

Components	Quantities
InhibiCore Rapid Plant PCR MasterMix (5X)	4µL
primer 1:	1µL (200nM (100 - 400 nM recommended)
primer 2:	1µL (200nM (100 - 400 nM recommended)
Probe	1µL (400nM (200 - 600 nM recommended)
Template DNA*	0.1 - 10 ng per reaction
Nuclease-free water	up to 20µL

FAST PCR conditions - 2 Step program

Step	Time	Temperature
Initial denaturation	**3 min**	**95 °C (important!)**
Denaturation	10 sec	95 °C
Annealing/Extension	30 sec	60 °C*
Number of Cycles	40	

Ordering Information and Contact

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