AMPLIQON
PCR ENZYMES & REAGENTS

Q-Extract DNA Extraction Solution combined with real-time PCR

Overview

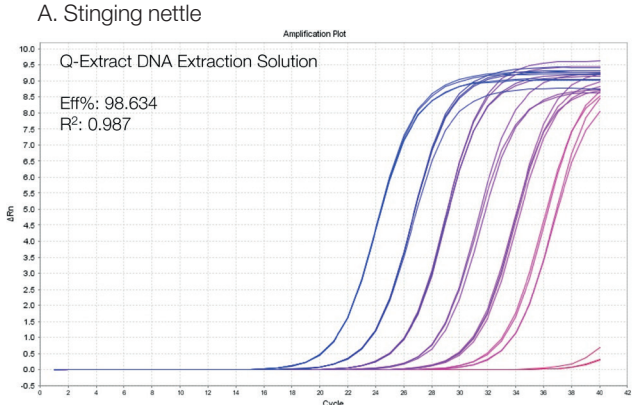
Q-Extract DNA Extraction Solution is designed for rapid and efficient extraction of PCR-ready DNA from various sample types; mammalian tissues (such as mouse tail, and ear snips), plant leaves, fish fins, saliva and bacteria. The non-toxic Q-Extract DNA Extraction Solution enables the extraction of PCR-ready DNA in just 8 minutes. The PCR-ready DNA are ideal for end-point PCR.

Here we describe how PCR-ready DNA extracted from stinging nettle (*Urtica dioica*) and ivy (*Hedera helix*) are used for successful real-time PCR using RealQ Plus 2x Master Mix Green, High ROX.

DNA extracted using Q-Extract DNA Extraction Solution is suitable for real-time PCR applications

Q-Extract DNA Extraction Solution was used to extract the PCR-ready DNA from leaves of stinging nettle and ivy. To examine the abillity of the PCR-ready DNA extracts to be used for real-time PCR applications the extracts were amplified using primers targeting chloroplast DNA (trnL 72 bp). For this experiment 5-fold serial dilutions of the extracted DNA were prepared. 7 dilutions (0.2 µl/ reaction) all in dublicate were included in the experiment (figure 1).

A. Stinging nettle



B. Ivy

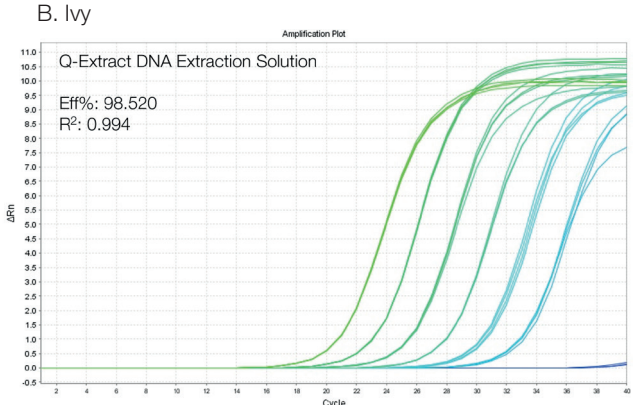


Figure 1. Data showing log amplification plots of 5-fold serial dilutions of stinging nettle and ivy all in dublicates. 7 dilutions (0.2 µl of each dilution/reaction) of each dublicate were included. A: Amplification plots of DNA extracted from stining nettle using Q-Extract DNA Extraction Solution (Ampliqon). B. Amplification plots of DNA extracted from ivy using Q-Extract DNA Extraction Solution (Ampliqon). Cycling was performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) with the following protocol: 95 °C, 15 min; followed by 40 cycles of 95 °C, 15 sec; 60 °C, 60 sec (table 3).

Conclusion

The results from this experiment show that it is possible to utilize the DNA extracted using Q-Extract DNA Extraction Solution for real-time PCR amplification with high efficeincy.

Table 1. Sample sizes of different matrices

	Q-Extract DNA Extraction Solution	
Sample	100 µl	500 µl
Tissue*	0.5 – 10 mg	10 – 50 mg
Plant**	2 – 10 mg	10 – 50 mg
E. coli	1 colony (Φ 0.5 - 2 mm)	1 colony (Φ 0.5 - 5 mm)
Saliva	10 – 20 µl	50 - 100 µl

* Examples of tested tissues include mouse tail snip, mouse organs and chicken breast.
**Examples of tested plant materials include leaves from stinging nettle and ivy.

Table 2. Real-time PCR reaction mix

Component	Volume	Conc.
Master Mix*	12.5 µl	1x
Forward primer (10 µl)	0.5 µl	0.2 µM
Reverse primer (10 µl)	0.5 µl	0.2 µM
PCR Grade Water	6.5 µl	-
DNA extracts (5-fold dilutions)	1 µl	0.2 µl/RXN
Total volume	25 µl	-

* RealQ Plus 2x Master Mix Green, High ROX

Table 3. 2-step real-time PCR protocol

Step	Temp.	Time	Cycles
Initial heating	95 °C	15 min	1
Denaturation Annealing/Elongation	95 °C 60 °C	15 sec 60 sec	40
Final elongation	72 °C	4 min	1
End	4 °C	∞	1

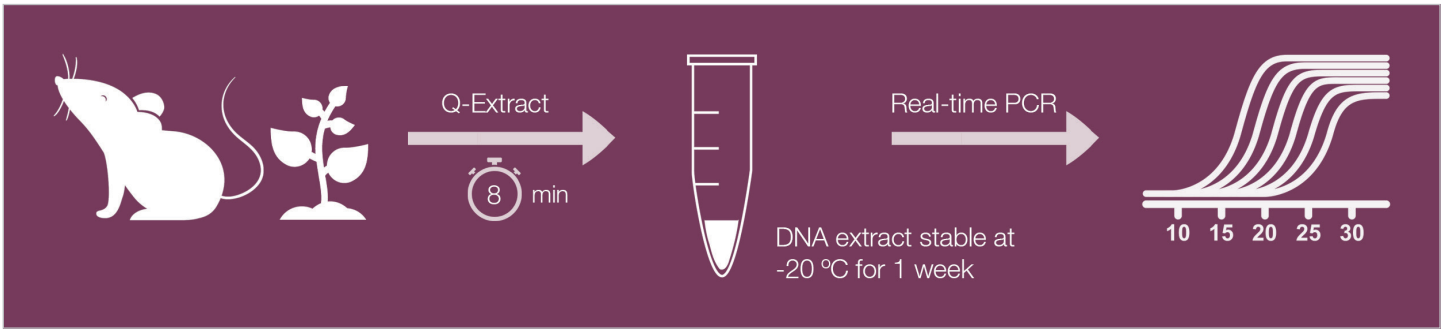
Q-Extract Extraction Protocol

1. Add ~10 mg of plant leaves* to a tube containing 100 µl Q-Extract DNA Extraction Solution. Make sure that the leaves are completely covered by the Q-Extract DNA Extraction Solution.
2. Vortex the tube for 15 sec.
3. 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 PCR.

DNA extracts are stable at -20 °C for one week or long-term storage at -80 °C.

*Recommended sample sizes from various sample types are shown in table 1.



Ordering information

Product	RXN*	Cat #
Q-Extract DNA Extraction Solution	100 500	A560001 A560004
Q-Extract DNA Extraction PCR Kit Incl. Taq DNA Polymerase 2x Master Mix RED	100 500	A570001 A570004
Q-Extract DNA Extraction Hot Start PCR Kit incl. TEMPase Hot Start DNA Polymerase 2x Master Mix A BLUE	100 500	A574401 A574404
SAMPLES: Q-Extract DNA Extraction Solution Q-Extract DNA Extraction PCR Kit Q-Extract DNA Extraction Hot Start PCR Kit	 20 20 20	 A560099 A570099 A574499

* 1 reaction = 100 µl Q-Extract DNA Extraction Solution + 12.5 µl Taq DNA Polymerase 2x Master Mix RED (final PCR reaction 25 µl)

Product	RXN*	Cat #
RealQ Plus 2x Master Mix Probe High ROX™	400 4000	A315402 A315406
RealQ Plus 2x Master Mix Probe Low ROX™	400 4000	A314402 A314406
RealQ Plus 2x Master Mix Probe Without ROX™	400 4000	A313402 A313406
RealQ Plus 2x Master Mix Green High ROX™	400 4000	A325402 A325406
RealQ Plus 2x Master Mix Green Low ROX™	400 4000	A324402 A324406
RealQ Plus 2x Master Mix Green Without ROX™	400 4000	A323402 A323406

* Final PCR reaction 25 µl

PCR ENZYMES MADE IN DENMARK

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