Manual

QuickExtract Plant DNA Extraction Solution

QuickExtract™ is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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1. Introduction

The QuickExtract Plant DNA Extraction Solution is a fast, simple and inexpensive method for preparing plant genomic DNA for PCR amplification. The DNA extraction requires only heat treatment to lyse the plant material, release the DNA and to degrade compounds inhibitory to amplification. Following heat treatment, sample DNA is ready for PCR.

The QuickExtract solution has been used for leaf or seed material to successfully extract PCR-amplifiable DNA from: *Arabidopsis*, hopvine, maize, grape, rosemary, rice, sunflower, cotton, rapeseed, wheat, tomato, pepper, soybean, spelt, and spinach. The gene targets included several single-copy and multiple-copy genes. Seeds should be cut, chipped, or ground. Leaf punches may be processed directly or ground. See Table 1.

QuickExtract Plant DNA Extraction Solution is provided at a 50 mL volume, sufficient for 500 (100 µL) extractions. Larger volume packaging and pricing are available upon request.

2. Product designations and kit components

Product	Kit size	Catalog number	Reagent description	Part numbers	Volume
QuickExtract Plant DNA Extraction Solution	50 mL (500 extractions)	QEP70750	QuickExtract plant extract	SS000773-D1	50 mL

Table 1.

3. Product specifications

Storage: Store at -20 °C in a freezer without a defrost cycle. Minimise the number of freeze/thaw cycles. Thawed QuickExtract solution can be stored at 4 °C for 1 month or refrozen in small aliquots.

Quality control: QuickExtract Plant DNA Extraction Solution is functionally-tested by assaying for the production of a PCR product from spinach leaf. A single-copy gene for heat shock protein HSP70 is used as the PCR template.

4. Tips for success

1. Plant species are notoriously diverse, which impacts sample preparation, DNA yield, and presence of debris and potential PCR inhibitors. Thus, it is important to empirically determine the best ratio of sample to QuickExtract Plant DNA Extraction Solution to get the most DNA yet minimise the release of undesirable cellular components. A good starting point is to use one to three leaf punches (4 mm to 6 mm diameter), or 10-100 mg of seed chip material per 200 µL of QuickExtract Plant DNA Extraction Solution for processing. Then determine which ratio results in the production of optimally amplifiable DNA, meaning reliable production of a strong expected PCR product, little background, minimal primer-dimer, etc.
2. Be certain enough QuickExtract Plant DNA Extraction Solution is used to sufficiently cover the sample and compensate for liquid absorption into the sample. A brief centrifugation may be required to pull the sample into the QuickExtract solution in the tube or well of a plate.

3. Grinding may be required for some plant materials. When grinding is used this will typically require a centrifugation step to pull down sample debris. Care must be taken to avoid aspirating the sample debris.
4. Plant tissues can release lignins, polyphenols and pigments which will inhibit PCR. Test a dilution series (1:4, 1:8, 1:16 and 1:32) of the processed sample to determine the best balance between DNA concentration and PCR inhibitors. When removing an aliquot from the extracted/processed sample, be careful to avoid aspirating the cellular debris. If possible, perform a quick centrifugation prior to aspirating your sample.
5. The second heating step at 95-98 °C may deform some plastics. If using microtiter plates, be sure that the plates you intend to use are suitable for this temperature.
6. It may be beneficial to add α-amylase to the QuickExtract Plant DNA Extraction Solution for some samples, especially seeds. Add 0.1 µg/µL α-amylase to the QuickExtract solution before processing.

5. High-throughput considerations

1. Because leaf punches can plug the tips of automated liquid handling devices, you may need to grind the leaf material to create a homogenous solution before processing or use a manual aspiration process.
2. When performing high-throughput applications, it may not be practical to perform the second heating step at 98 °C, as some microtiter plates will warp at 98 °C. This step may be performed at 95 °C. Be sure that the plates you intend to use are suitable for this temperature.
3. When adding the QuickExtract Plant DNA Extraction Solution to the sample, it should be added above the well so that the same tips can be used to dispense multiple plates without cross-contamination.
4. Be sure to use a sufficient volume of QuickExtract Plant DNA Extraction Solution to enable safe removal of processed sample without aspirating debris.
5. If using a water bath that holds plates on side, care needs to be taken to ensure the QuickExtract Plant DNA Extraction Solution is covering the plant material.
6. If the extracted samples are dispensed later with a liquid handler, we recommend watching for pipetting issues. It has been reported that corn seed extracted samples caused bubbles to form in the tips during tip conditioning leading to dropouts. This was resolved by making the pipette wash more stringent and limiting the conditioning step.

6. Protocol

Note: Refer to the "Tips for success" section above before proceeding.

1. For Leaves: Cut a 4-6 mm diameter leaf disc using a leaf punch. Note, if processing just a few samples you may use the cap of a 500 µL microfuge tube to capture the leaf section (simply snap the tube closed over the portion of the leaf to be sampled). For Seeds: Use 10-100 mg of seed chips or ground seed material as appropriate. See Table 2.
2. Place the leaf disc (or the seed material) into a 500 µL tube or a well of a 96-well plate, add 100 µL of QuickExtract Plant DNA Extraction Solution and immerse the tissue.
3. Heat the samples at 65 °C for 6 minutes then at 98 °C for 2 minutes.
4. Place extracted samples at 4 °C for short term storage or -20 °C for long term storage. Samples may need to be diluted prior to PCR. See "Tips for success".

7. Troubleshooting

1. Plant lignins, polyphenols, pigments, etc. which will be in the extract may be inhibitory to amplification. If the PCR is unsuccessful using undiluted extract, test a dilution series (1:4, 1:8, 1:16 and 1:32) of the QuickExtracted sample to determine the best balance between DNA concentration and PCR inhibitors. While it may be counterintuitive to use less starting DNA material, better results are sometimes achieved by using less processed sample thereby reducing the amount of potential PCR inhibitors in the reaction.
2. Determine whether grinding is required, or is counterproductive. See Table 2.
3. Optimisation of the PCR may be necessary. Follow common practices for optimisation (titrate magnesium concentration, adjust annealing and/or elongation temperature and time, add PCR Enhancers). Alternatively the FailSafe™ PCR PreMix Selection Kit (Cat. No. FS99060) provides a quick optimisation procedure to increase success rates.

Crop	Tissue type	Process-ing	Grinding	Amount	QuickExtract Buffer added (µL)
Corn	Seed	Chipped	Optional	~20 mg	200
				~100 mg	400
	Leaf	Punches*	Optional	1-2 punches	100
				5 punches	200
				20 punches	400
Wheat	Seed	Whole	Required	~35 mg	200
	Leaf	Cut	Required	~5-10 mg	100
				~20 mg	200
				~45 mg	400
Rapeseed	Seed	Whole	Required	~5 mg	100
	Leaf	Punches	Grinding not tested	~5-10 mg	100
Tomato	Seed	Whole	Required	~3 mg	100
	Leaf	Punches	Optional	5 punches	200
				15 punches	400
Pepper	Leaf	Punches	No Grinding	5 punches	200
				15 punches	400
Cotton	Seed	Whole	Required	~100 mg	200
		Hulled	Required	~50 mg	200
	Leaf	Punches	Not tested	2 punches	200
Sunflower	Seed	Whole	Required	~100 mg	200
		Hulled	Required	~50 mg	200
	Leaf	Punches	Not tested	2 punches	200

Table 2. Sample and pretreatment of validated crops, seeds or leaves, grinding requirement conditions, starting weight, and volume of required QuickExtract solution. The guidelines show how much tissue to use, and whether grinding the sample was required for each plant species and tissue type. For reference, one punch is 6 mm in diameter. Additional optimisation may be required.

*use a leaf punch which produces a 4-6 mm disc.

8. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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