

Frequently Asked Questions (FAQs)

About the SYBR® Green Extract-N-Amp™ Plant PCR Kit

What reagents do I need to provide to use this method?

None. All reagents for extraction and PCR are included in the SYBR Green Extract-N-Amp Plant PCR Kit. The only reaction components missing are your gene specific primers and an internal reference dye if your thermal cycler requires one.

Can I use plant tissue other than leaf in the protocol?

The kit protocol has been optimized for leaf tissue. While some customers have used the kits successfully for other tissue types, the kits are not intended for tissue types other than leaf.

Can DNA extracted with the SYBR Green Extract-N-Amp Plant PCR Kits be used in applications other than PCR – such as restriction digestion or Southern blots?

Since the kits do not purify genomic DNA, applications other than PCR are not intended.

Will the kit reagents work with any real-time thermal cycler?

The PCR components are not optimized to work with capillary-based real-time thermal cyclers, such as the Roche LightCycler®. However, any plate-based real-time thermal cyclers such as models from ABI, Stratagene and Bio-Rad (including former MJ Research models) are 100% compatible with the SYBR Green Extract-N-Amp Plant PCR Kits.

Do I need to add a reference dye to the PCR reaction?

Reference dye is not included with the SYBR Green Extract-N-Amp Plant PCR Kits. Not all brands of thermal cyclers require the use of an internal reference dye. Refer to your thermal cycler manufacturer's instruction manual to determine the type and concentration of reference dye appropriate for your instrument and experimental goals.

What size targets are appropriate for amplification?

According to the book, *A-Z of Quantitative PCR*, edited by Stephen A. Bustin, 2004, "Two main factors to consider when optimizing efficiency in real-time PCR experiments are amplicon size and primer placement. Amplicons in real-time PCR reactions should be designed to be 100-150 bp or less. The shorter the amplicon, the higher the amplification efficiency tends to be." While the kit protocol allows for amplicons up to 500 bp, primers should be designed for the smallest amplicon that allows for conclusive experimental results.

Can I optimize my PCR conditions?

Yes. You could adjust your PCR reaction knowing that the available magnesium in a 20 µl PCR reaction is 3 mM, but this may interfere with the optimal performance of the kit. For advice and information on optimal reaction conditions, contact Sigma Technical Services at **1-800-325-5832** or by email at techserv@sial.com.

How long can the extracted DNA be stored?

Extracts from many species are stable at 4 °C for at least 6 months. However, -20 °C is recommended for long-term storage or until it is determined that your samples are stable at 4 °C.

I want to include a positive control sample (purified genomic DNA) with my extracted samples for PCR. I would also like to dilute some of my extraction samples before PCR.

Can I use water for these purposes?

No. Water will significantly alter the ionic balance of the PCR reaction. For positive controls or dilutions, use the Extract-N-Amp PCR Diluent (Product Code E8155, not included in kits) or a 50:50 mixture of the Extraction and Dilutions solutions included in the kits.

Should the plant leaf sample completely dissolve in the extraction solutions?

No. The SYBR Green Extract-N-Amp Plant PCR Kit was developed to extract sufficient DNA for PCR from plant cells through a quick 15-minute procedure. Typically, there is little to no visible degradation of the leaf tissue.

Is it possible to use the same plant sample twice for extraction and amplification?

After extracting genomic DNA from a plant tissue sample using the SYBR Green Extract-N-Amp Plant PCR Kit, the sample can be removed from the extraction solutions, rinsed and used again (with a fresh aliquot of reagents) to extract genomic DNA using the same kit procedure.

Is it possible to use a genomic DNA purification kit or protocol on a tissue sample that I have already used in the SYBR Green Extract-N-Amp Plant method?

Yes. Since the SYBR Green Extract-N-Amp Plant PCR Kit does not purify genomic DNA or significantly degrade leaf tissue, the leaf sample can be removed from the kit extraction solutions, rinsed, and used in a genomic DNA purification method.

What if I want to perform more than one PCR reaction on a single sample?

The SYBR Green Extract-N-Amp Plant PCR Kits contain reagents sufficient for one extraction and one PCR reaction per tissue sample. However, the extraction protocol yields enough volume for up to 50 20 µl PCR reactions. For experiments where multiple PCR reactions per sample are required, additional SYBR Green PCR ReadyMix™ (Product Code S4320) can be purchased separately.

Can RNA from the leaf tissue samples be separated or used for gene expression experiments?

No. RNA is degraded during the DNA extraction procedure.

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