Spectrum Plant Total RNA Kit

Frequently Asked Questions (FAQs)

I've tried other kits with protocols that seem very similar to the Spectrum protocol and those kits gave me very poor results with my tissue sample. What is different about the Spectrum Kit that I should consider?

If the format of the protocol seems familiar, you have probably used one of the many kits for RNA purification that are commercially available. Most of these kits utilize a lysis reagent for releasing total RNA from cells and a binding column which allows for anchoring of the RNA. Binding columns are typically compatible with centrifugation, allowing for quick passage of reagents across the column. This general format is very quick and very convenient.

In designing the Spectrum Kit, we decided to use this format and retain the benefits of speed and ease. The significant difference then, between the Spectrum Plant Total RNA Kits and all other kits, is the chemistry. The chemistry that Sigma developed is currently patent-pending and is a result of our specific goal of engineering a fast and easy total RNA purification kit that yielded high quality total RNA from plant tissues with high levels of secondary metabolites.

What is this chemistry and how does it differ from other protocols?

The chemistry that was developed works, not only to lyse cells and protect RNA from degradation, but also to remove secondary metabolites and enhance binding to the column substrate. Our research data indicated that the ability to remove secondary metabolites from the sample before column binding had a direct effect on the quality and yield of RNA. We developed a protocol that efficiently removes polysaccharides, polyphenolics, tannins, flavanoids, and many other compounds before the binding step, and we accomplished this in the framework of a column-based 'bind-and-elute' method that protected the RNA from degradation and increased yields of total RNA. The result is highly purified high quality total RNA suitable for nearly any application.

What applications have been validated for use with total RNA purified by the Spectrum Kit?

cDNA synthesis, Northern blotting, RT-PCR and qRT-PCR have all been performed using RNA purified by the Spectrum Kit and the resulting data from all of these applications matched theoretical results. Other applications such as nuclease protection assay, *in vitro* translation and epitope tag labeling have not been performed at Sigma, however all available data strongly suggests that Spectrum-purified RNA is perfectly suitable for use in these applications.

I don't see the species or tissue type I work with as validated. Does that mean the Spectrum Kit won't work for me?

Absolutely not! Because of the large diversity of plant species that are being researched, it is impossible for Sigma Scientists to validate every species and tissue type. Because of out geographic location (Saint Louis, Missouri, USA), there is also a limitation to what fresh plant material we can access.

As we get feedback from researchers who independently validate the Spectrum Kit on new species and tissues we add those to the validated list. Therefore the list is frequently updated. However, we engineered the Spectrum Plant Total RNA Kit to work with some of the most notoriously difficult sample types such as pine needles, potato tubers, citrus fruit skin and cotton fibers. Successful purification of total RNA from these samples suggests that any other samples of an equal or lesser difficulty would also be successful.

If you would like to help validate a new sample type or connect with another researcher who may be using the Spectrum kit for a similar purpose, contact us at plant@sial.com.

Does the protocol purify all of the total RNA present in a tissue sample?

Our data indicates that small RNA, such as microRNA, siRNA, tRNA and 5S rRNA is not efficiently recovered using the standard kit protocol. However, if desired, there is an alternate protocol that is included with the Kit instructions that outlines how to increase the efficiency of small RNA recovery. No additional components are necessary.

How is the integrity of the RNA maintained through the purification process?

Endogenous RNases are denatured during the lysis step of the protocol. Secondary protection is offered by beta-mercaptoethanol that is added to the Lysis Solution which reduces RNase disulfide bonds causing disruption of the protein's folding structure. Denatured RNases are subsequently removed during column purification. Despite these features, care must be taken to avoid introducing exogenous RNases during RNA preparation, especially during the final wash and elution steps. RNases are ubiquitous and very stable enzymes and generally do not need cofactors for enzymatic activity.

Can I purify >100 µg of total RNA if I increase the amount of plant tissue I start with?

The column substrate cannot bind more than 100 µg of total RNA and therefore this mass is the maximum theoretical yield for a single purification. It is important to note that deviations from the protocol instructions in any manner will most likely cause column clogging, reduced yield, incomplete purification or failure of the complete system. The kit protocol was optimized and validated extensively to ensure the best performance with the largest number of plant species.

Can I purchase kit components separately of the kit?

Currently, individual kit components are not available for sale as they are validated as part of a system and their performance cannot be guaranteed in unknown applications. However, if the largest kit size is significantly smaller than you require, a larger custom packaging configuration may be available. Inquires may be emailed to plant@sial.com.

Can I reuse the binding columns?

The binding columns are not intended for use with more than one sample. We have no recommendations for "recharging" or "reconditioning" the columns. While the greatest risk of reuse is cross-contamination between two different total RNA populations, there is also no way to guarantee that the columns would bind and/or elute a suitable amount of RNA.

Is there a way to fractionate the DNA, proteins or chemical compounds that are removed during the purification process?

Fractionation of cellular contents is not possible with this protocol.

What about other questions?

The Sigma Technical Services Department is always available to help you with technical issues and questions. These scientists can be reached at 1-800-325-5832 (US and Canada) or online at sigmaaldrich.com/techservice (Global).