spex® sampleprep

App Note

The Process of DNA Extraction: Using Spex DNAmax DNA Extraction Kits for Effective DNA Extraction and Isolation

The extraction of nucleic acids (DNA and RNA) is a common practice in molecular biology. The processes used for nucleic acid isolation often result in purified genetic material that can be used in a variety of applications, such as the polymerase chain reaction (PCR) and quantitative PCR (qPCR). These techniques often depend on the purity and quality of the DNA extracted from sample biological tissue in order to produce accurate results. It cannot be understated that the processing of biological tissues prior to DNA isolation plays a critical role in the final result.

In DNA extraction there are three basic steps: 1. tissue homogenization and cell lysis; 2. DNA precipitation and extraction; and 3. DNA isolation and purification.

DNA Extraction Steps

Step 1: Tissue Homogenization and Cell Lysis

In the first step, the tissue is homogenized into an analytical form which reduces particle size, disrupts cell walls or lipid bilayers, and ruptures the nuclear envelope to facilitate the release of the genetic material. Some types of tissues require mild homogenization and lysis due to their non-fibrous nature while other tissues, such as plant material, need vigorous disruption due to their rigid cell walls. There are generally three methods for cell lysis: 1. mechanical or physical disruption; 2. chemical lysis (including enzymatic lysis); and 3. combination methods.

1. Physical or mechanical methods involve some type of grinding apparatus to disrupt the tissue and break apart cell walls or lipid bilayers. These methods can range from simple manual methods including mortar and pestle to more efficient automated laboratory homogenizers and grinders. These methods work on a variety of tissues contained in hard-to-digest tissues category which resist cell lysis such as plant material, bone and fibrous samples.

2. Chemical methods by themselves can be used on easy-to-digest materials such as monolayer cells or cells in suspension where the impact of a physical disruption is not essential for lysis. The cellular disruption is facilitated by a number of agents that break down cell membranes or denature proteins, such as chaotropes like guanidine salts and alkaline solutions and/or detergents.

   Enzymatic methods are a type of chemical-based method for lysis employed on structured materials (plants, yeast, etc.) and is often used with other types of disruption techniques. Enzymatic treatments for cell lysis depend upon the type of tissue and target different chemical modalities and structures. Typical enzymes used to lyse compounds include lysozyme, proteinase K, lipase, and others.

3. Many lysis protocols combine different approaches for the complete disruption of the cells in the sample and may be used sequentially or simultaneously in a protocol. The goal of all the lysis protocols is to break apart the structures of the cell to release the genetic material. Other components may be added to the sample (such as salts) to stabilize the DNA during lysis.

Step 2: Precipitation and Extraction

After cellular lysis and proteinase K digestion of histones, DNA gets released into the slurry of cellular debris, plant or mammalian secondary metabolites and proteins from which it needs to be separated. Salt ions, such as sodium, are added to the slurry to stabilize the DNA molecule and neutralize the negative charge on the phosphodiester backbone. This results in a more stable and less water-soluble molecule. Alcohols, such as ethanol or isopropanol, are then added to the slurry which alters the dielectric constant of water, thereby forcing the DNA to precipitate from the cell debris.

Step 3: Isolation and Purification

After the DNA has been precipitated from the cellular debris, it is then purified by a series of wash steps. Alternatively, one can use silica spin columns which bind the DNA to the column, allowing for the washing of the cellular debris from the sample. The DNA is retained on the positively charged silica column and the remaining material is discarded as waste. The DNA material can then be washed, further dried, or resuspended for further processing. The purity of the sample can be confirmed by a variety of methods including gel electrophoresis (Figure 1) or UV-VIS spectroscopy (Figure 2a and 2b).

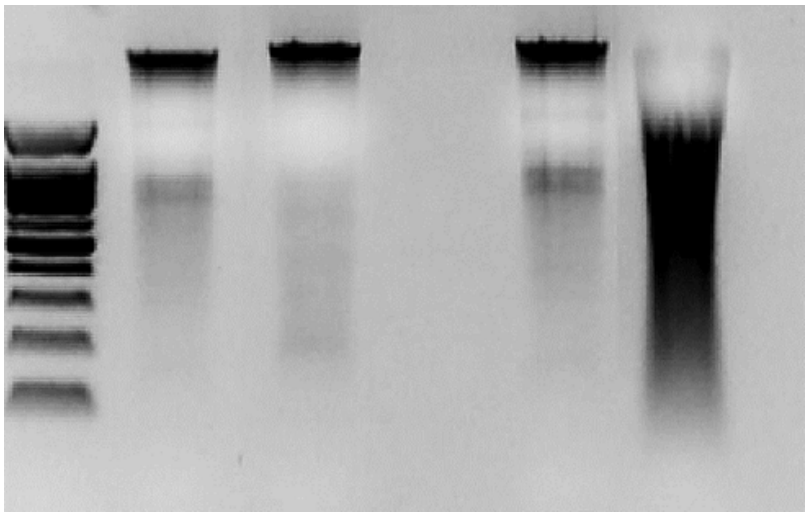


Figure 1. Examples of DNA extraction purity by gel electrophoresis.

Figure 2a

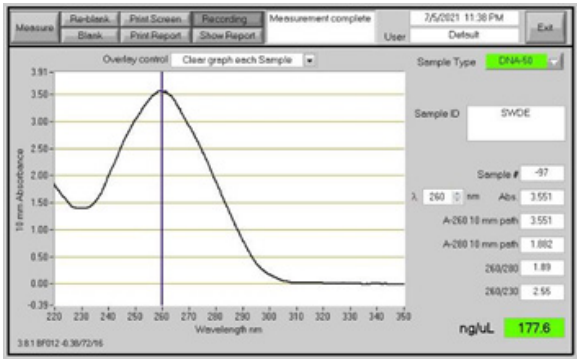


Figure 2b

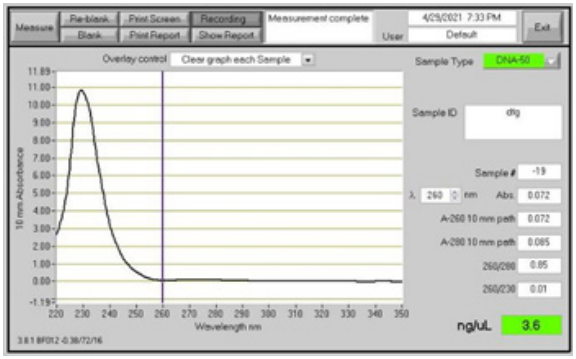


Figure 2. Examples of DNA extraction purity by spectrophotometry where (a) shows absorbance at 260 nm indicating pure DNA where (b) shows no absorbance at the DNA wavelength of 260 nm.

Using Spex DNAmax Extraction Kits

The exact protocol followed often depends upon the type of tissue being extracted. Some animal and plant tissue can be difficult to homogenize and extract and requires different protocols to process successfully.

All Spex DNAmax kits are designed to have all the supplies and materials needed for the optimized DNA extraction (except application specific buffers and solvents, see protocols for exceptions). Plant and animal tissue kits both contain sample type appropriate reagents including lysis, wash and elution buffers, in addition to RNase A and proteinase K. Optional stabilization buffer is offered for the animal tissue kits. Plant kits contain an additional wash buffer.

Supplies in all kits include a written comprehensive protocol designed for Spex homogenizers. The kit also includes the following supplies: DNA binding columns, collection tubes, grinding media, and homogenization tubes all optimized for the type of tissue being extracted (Figure 3).

Figure 3. Example of Spex DNAmax Extraction Kit contents.

Comparison of Spex DNAmax Kits to Competitor Products

Spex DNA extraction kits are some of the most versatile and complete kits on the market allowing for maximum throughput with all extraction components at an affordable cost. DNAmax provides 25 more reactions per kit than the leading competitors and offers kits for both plant and animal tissue (with and without stabilizers) not found with competitors; all at a price significantly lower than other kits (Table 1).

Table 1. Comparison of DNAmax kits against competitors.

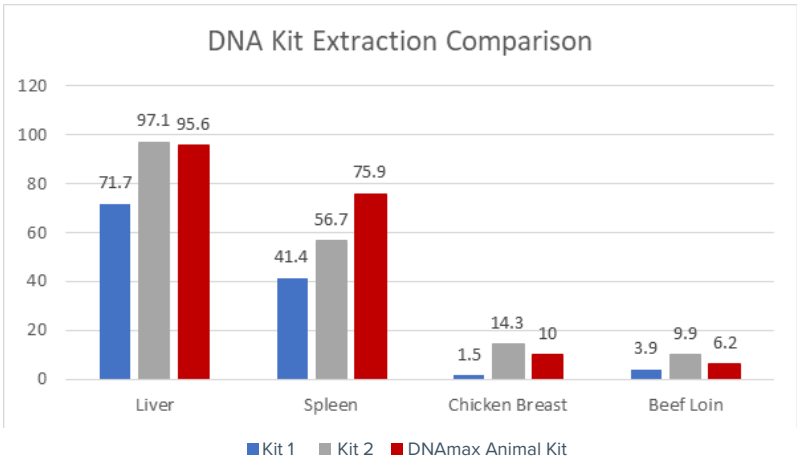
Features	DNAmax	Competitor A	Competitor B
# Samples	75	50	50
Stabilizer	✓*	✗	✗
Plant Kits	✓	✓	✓
Animal Kits	✓	✓*	✗
Average $/ Sample	<$3.20	$3.65	$5.50
Optimized for Genomax	✓	✗	✗

* Optional

Spex DNAmax Kit Results for Animal Tissue

The Spex DNAmax kits perform very well against competitor products for animal tissue DNA extraction and isolation. Our studies show high amounts of DNA recovered using DNAmax kits which are better or equivalent to the responses of competitor kits (Table 2).

Table 2. DNA recovery from Spex DNAmax animal tissue extraction kits.



Tissue such as liver and spleen displayed a high quantity of purified DNA well in range of other kits with improved purity and less degradation (Figure 4).

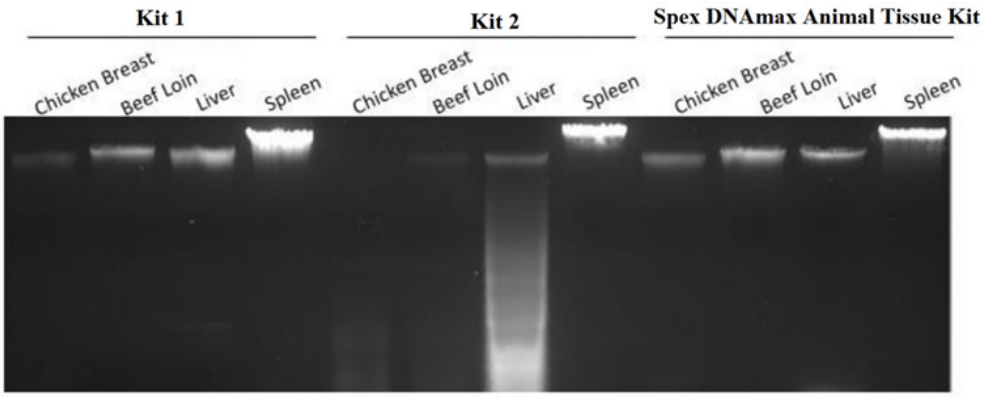
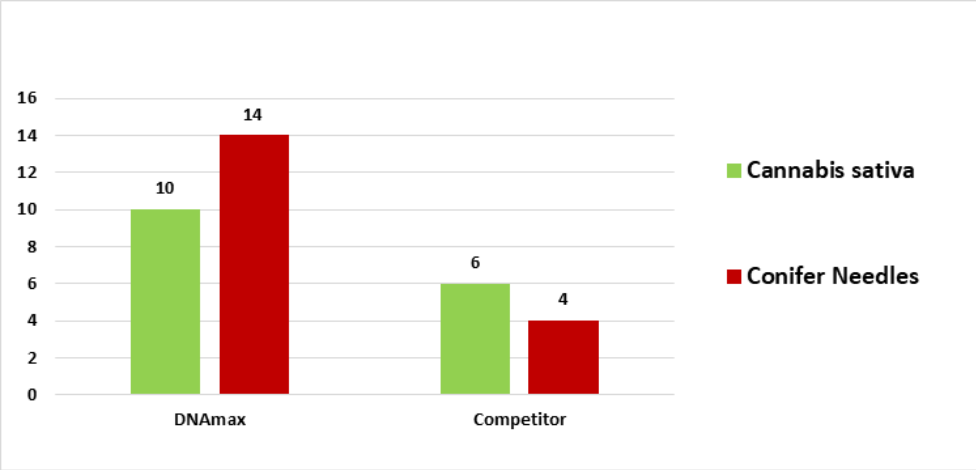


Figure 4. Gel electrophoresis comparison of animal tissue using Spex DNAmax kits versus competitor kits.

Spex DNAmax Kit Results for Plant Tissues

Spex DNAmax kits for plant tissues used in conjunction with the included grinding media and homogenized by a Spex homogenizer were found to be very effective at extracting all types of DNA. Quantities of DNA extracted from cannabis and conifer needles were higher in samples using the DNAmax kits versus the competitor (Table 3). The quality of the DNA was also found to be better using the Spex DNAmax kits (Figure 5).

Table 3. DNA extracted using Spex DNAmax plant tissue kit versus a competitor.



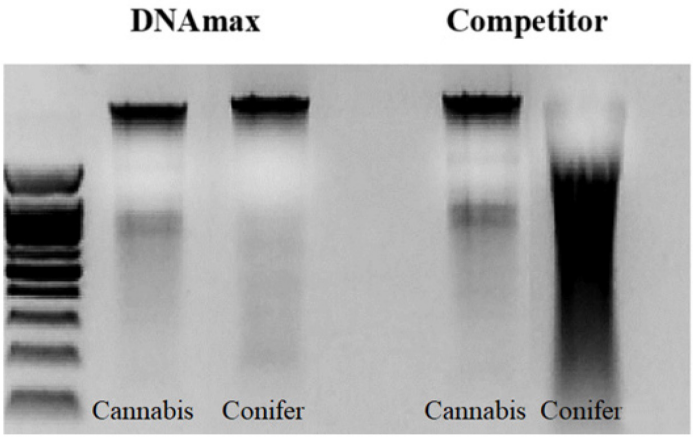


Figure 5. Gel electrophoresis of plant DNA from DNAmax and competitor isolation kits.

Further PCR on the universal plant *rbcL* gene displayed excellent amplification in many types of plant materials (Figure 6).

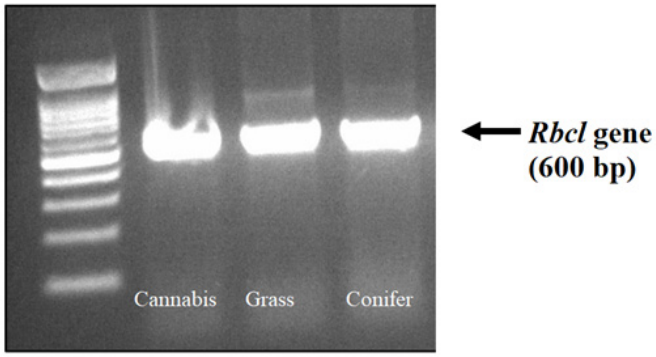


Figure 6. PCR amplification of the universal plant *rbcL* gene using DNA from various plant tissues isolated using the Spex DNAmax kit.

Conclusions

The Spex DNAmax extraction kits were compared with other competitors' kits based on the criteria of DNA recovery, efficiency, quality of DNA recovered, versatility, and value. In all categories, the Spex kits performed either better than or equal to competitive kits. Spex kits can be used seamlessly and most efficiently with predeveloped protocols intended for Spex homogenization products such as the Genomax®, Geno/Grinder® or GenoLyte®; but can also be used with all common laboratory homogenization protocols. Protocols for the most common tissue types are included in the Spex kits. In addition, Spex kits have been shown to be compatible with a wide variety of tissues, cell cultures and suspensions. In the categories of versatility and value, Spex kits perform higher with more reactions per kit, greater kit choice flexibility and lower costs.

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