



# PHYTOPURE<sup>®</sup> GENOMIC DNA FROM PLANT TISSUES

## Principle steps in the Nucleon<sup>®</sup> PhytoPure protocol

Breaking of Cell Wall

Cell Lysis with Potassium/SDS

DNA Extraction with Nucleon  
PhytoPure Resin and Chloroform

DNA Precipitation

DNA Washing

- High speed (<60 minutes)
- High yield ( $\geq 60\mu\text{g}/1\text{g}$  plant)
- High purity ( $A_{260}/_{280} > 1.8$ )
- High MWt DNA extraction (>50kb)
- Patented chemistry to ensure polysaccharide-free DNA preparations
- Fully scalable (0.1g-1.0g)

### Introduction

The Nucleon PhytoPure system for extracting DNA from plant samples is capable of producing high yields of high quality DNA in a fraction of the time taken by conventional methods. Not only is it more efficient, but it is considerably simpler.

Polysaccharides are common contaminants in plant DNA extracts often resulting in difficult to handle, 'slimy' DNA pellets. A particular problem are those of an anionic nature which can be inhibitory to further enzymatic analysis of the DNA. The Nucleon PhytoPure DNA extraction system has been developed specifically to solve these problems.

### Principle

After breaking of the cell wall, the cells are lysed in a reagent containing potassium/SDS which is known to complex with proteins and polysaccharides. Chloroform is then added along with the Nucleon

PhytoPure proprietary resin. This resin performs two vital functions during the extraction process. It covalently binds polysaccharides with free  $-\text{B}(\text{OH})_2$  groups on its surface (Figure 1), thus removing them from the sample. Additionally, the resin forms a semi-solid stratum during partitioning (Figure 2) with chloroform which facilitates DNA recovery, ensuring high yields of high quality DNA.

### Applications

DNA recovered by the Nucleon PhytoPure DNA extraction system is suitable for restriction digests (Figure 3), PCR<sup>†</sup> (Figure 4), blotting and hybridisation.

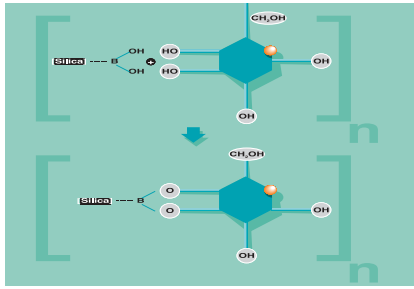
Nucleon PhytoPure has been used successfully on a wide range of plant species (Table 1) and the list is still growing. DNA can be extracted from fresh, frozen or freeze-dried material. The kit is available in two sizes and the protocols can accommodate large and small sample preparations.



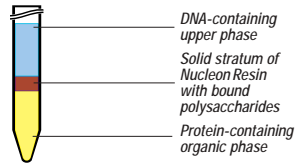
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**Figure 1 Polysaccharide binding mechanism**



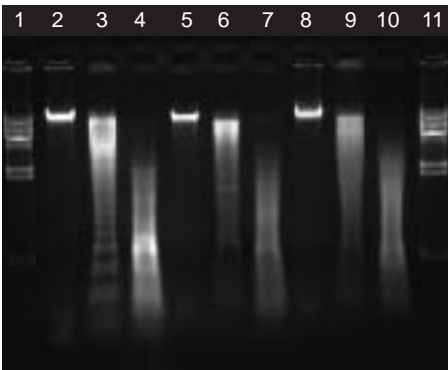
**Figure 2 Formation of semi-solid stratum after adding Nucleon Resin and chloroform**



**Table 1 Species from which DNA has been extracted using Nucleon PhytoPure**

<i>Arabidopsis thaliana</i>	<i>Musa spp</i> (banana)
<i>Araucaria araucana</i> (Monkey puzzle tree)	<i>Malus spp</i> (apple)
<i>Beta vulgaris</i> (sugar beet)	<i>Nicotiana tabacum</i> (tobacco)
<i>Brassica oleracea</i> (cabbage family)	<i>Phaseolus vulgaris</i> (red kidney bean)
<i>Brassica napus</i> (oilseed rape)	<i>Pinus sylvestris</i> (Scots pine)
<i>Capsicum annuum</i> (chilli pepper)	<i>Pisum sativum</i> (pea)
<i>Capsicum frutescens</i> (sweet pepper)	<i>Rhododendron spp</i>
Cereals (barley, maize, rye, wheat)	<i>Salix spp</i> (willow)
<i>Cocos nucifera</i> (coconut)	<i>Solanum tuberosum</i> (potato)
<i>Crytomeria</i> (Japanese cedar)	<i>Sorghum</i> seeds
<i>Eucalyptus globulis/grandis</i>	<i>Sphagnum</i> (bog moss)
<i>Fragaria x ananassa</i> (strawberry)	<i>Spinacea oleracea</i> (spinach)
<i>Fucus</i> (brown seaweed)	<i>Swietenia macrophylla</i> (mahogany)
<i>Gossypium hirsutum</i> (cotton)	<b>Filamentous Fungi:</b>
<i>Helianthus annuus</i> (sunflower)	<i>Aspergillus niger</i>
<i>Helianthus tuberosus</i> (Jerusalem artichoke)	<i>Mortierella alpina</i>
<i>Heavea braziliensis</i> (rubber)	<i>Colletotrichum gloeosporioides</i>
<i>Humulus lupulus</i> (hop)	<i>Septoria nadorum</i>
<i>Irvingia gabonensis</i> (mango)	<b>Nematode:</b>
<i>Lolium</i> (ryegrass)	<i>Rhizopholus similis</i>
<i>Lotus japonicus</i>	<b>Slugs</b>
<i>Lupinus albus</i> (Lupin)	<b>Snails</b>
<i>Lycopersicon esculentum</i> (tomato)	

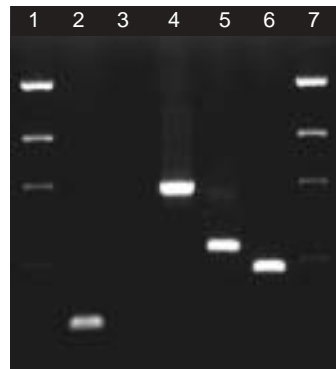
**Figure 3 Genomic DNA isolation and restriction enzyme digests**



DNA, isolated from Arabidopsis, Maize and Onion, using the Nucleon protocol, was digested with *HindIII* and *AluI* restriction enzymes and analysed on a 1.0% agarose gel.

- 1) *HindIII*  $\lambda$  digest marker
- 2) Undigested DNA purified from Arabidopsis
- 3) DNA purified from Arabidopsis digested with *HindIII*
- 4) DNA purified from Arabidopsis digested with *AluI*
- 5) Undigested DNA purified from Maize
- 6) DNA purified from Maize digested with *HindIII*
- 7) DNA purified from Maize digested with *AluI*
- 8) Undigested DNA purified from Onion
- 9) DNA purified from Onion digested with *HindIII*
- 10) DNA purified from Onion digested with *AluI*
- 11) *HindIII*  $\lambda$  digest marker

**Figure 4 PCR amplification**



DNA, isolated from Arabidopsis, Maize and Onion, using the Nucleon protocol, was used as a template for PCR amplification using primers corresponding to the plant chloroplast *Taberlet* region. The products were separated on a 1.0% agarose gel.

Low DNA Mass Ladder, Invitrogen (Cat#10068-013) {2000, 1200, 800, 400, 200 and 100 bp}

## Ordering Information

**SL8510** Nucleon PhytoPure kit for 50 extractions of 0.1g of plant tissue

**SL8511** Nucleon PhytoPure kit for 50 extractions of 1.0g of plant tissue

Other kits available in the Nucleon range:

- SL8501** Nucleon BACC1 kit for 50 extractions of up to 1ml whole blood or cell cultures
- SL8502** Nucleon BACC2 kit for 50 extractions of between 3 to 10ml of whole blood or cell cultures
- SL8512** Nucleon BACC3 kit for 50 extractions of up to 10ml of whole blood or cell cultures
- SL8508** Nucleon ST kit for 50 preps of up to 250mg of soft tissue
- SL8509** Nucleon HT kit for 50 preps of up to 25mg of hard tissue or paraffin-embedded sections
- 44100** Nucleon Genomic DNA Extraction kit for 50 preps of 10ml whole blood
- 44200** Nucleon Genomic DNA Extraction kit for 50 preps of 1cm mouse tail
- 44201** Nucleon Genomic DNA Extraction kit for 200 preps of 1cm mouse tail

- 44300** Nucleon Genomic DNA Extraction kit for 50 extractions of 0.1g of plant tissue

Other kits available in the Nucleopure™ range for use with the Nucleopure Automated System for mouse tail DNA extraction:

- 35100** Nucleopure Mouse Tail kit for up to 960 extractions

Other kits available in the Nucleoplex™ range for use with the Nucleoplex Automated System for BAC/Plasmid DNA extraction:

- 33100** Nucleoplex Plasmid DNA extraction kit for up to 192 extractions
- 33200** Nucleoplex BAC DNA extraction kit for up to 192 extractions
- 33201** Nucleoplex BAC DNA extraction kit for up to 192 extractions (plasticware not included)



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