

## WARRANTY

Nucleon kits come with a 'no quibble' warranty. We trust that this Nucleon extraction kit will perform to your satisfaction but should you have any problems or technical enquiries please contact your local supplier.

### Kits available in the Nucleopure™ range:

<b>35100</b>	Nucleopure Mouse Tail kit for up to 960 extractions
<b>35200</b>	Nucleopure QD Mouse Tail kit for up to 960 extractions

### Kits available in the Nucleoplex™ range:

<b>33100</b>	Nucleoplex Plasmid kit for up to 192 extractions
<b>33200</b>	Nucleoplex BAC kit for up to 192 extractions
<b>33201</b>	Nucleoplex BAC kit for up to 192 extractions (plasticware not included)

### Kits available in the Nucleon range:

<b>SL8501</b>	Nucleon BACC1 kit for 50 extractions of up to 1mL whole blood or cell cultures
<b>SL8502</b>	Nucleon BACC2 kit for 50 extractions of between 3 to 10mL of whole blood or cell cultures
<b>SL8508</b>	Nucleon ST kit for 50 preps of up to 250mg of soft tissue
<b>SL8509</b>	Nucleon HT kit for 50 preps of up to 25mg of hard tissue or paraffin-embedded sections
<b>SL8510</b>	Nucleon PhytoPure® kit for 50 extractions of 0.1g of plant tissue
<b>SL8511</b>	Nucleon PhytoPure kit for 50 extractions of 1.0g of plant tissue
<b>SL8512</b>	Nucleon BACC3 kit for 50 extractions of up to 10mL of whole blood or cell cultures
<b>44100</b>	Non-chloroform Blood kit for 50 preps of 10mL whole blood
<b>44200</b>	Non-chloroform Mouse Tail kit for 50 preps of 1cm mouse tail
<b>44201</b>	Non-chloroform Mouse Tail kit for 200 preps of 1cm mouse tail
<b>44300</b>	Non-chloroform Plant kit for 50 extractions of 0.1g of plant tissue

Please contact your local supplier for further information.

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†PCR is a process covered by patents owned by Hoffman La-Roche and use of this process may require a licence.

# NUCLEON® GENOMIC DNA EXTRACTION KIT

## PHYTOPURE®

**Product number: SL 8510 and SL 8511**

For 50 extractions of 0.1g and 1.0g plant tissue

**NB: Please refer to kit contents section for storage instructions**



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Nucleon PhytoPure kits, employing the patented PhytoPure Resin, have been used by scientists for many years and have consistently provided high yields of highly pure, largely intact DNA from a range of sample types.

Whilst most plant DNA extraction techniques are effective in removing proteins, they are much less successful with polysaccharides. Polysaccharides are very common contaminants in plant DNA extracts, and often result in difficult-to-handle, 'slimy' DNA pellets. This problem is compounded as polysaccharides, particularly those of an anionic nature, can be inhibitory to further enzymatic analysis of the DNA.

PhytoPure Resin utilises a unique solid phase chemistry to ensure polysaccharide-free DNA preps. The proprietary resin binds unwanted cell impurities but does not bind the DNA - unlike most other bind/elute protocols available. This optimises the recovery yields of DNA.

**Efficiency:** The DNA is not bound to a solid surface at any point in the process. This avoids exposing the DNA to shearing forces and promotes the recovery of high yields of highly intact DNA.

**Purity:** The recovered DNA is free from impurities that might affect downstream applications and consistently provides  $A_{260/280}$  ratios of  $\geq 1.8$ .

**Ease of use:** The chemistry is highly robust and consistent in operation and through the use of PhytoPure resin is designed to maximise recovery whilst minimising carry-over of impurities.

**Safety:** This kit generates no phenol waste by-products.

### TYPICAL YIELDS

This kit operates most effectively over the sample weight range 0.1g-1.0g. Yields are typically  $>60\mu\text{g}/1\text{g}$  plant tissue. The DNA has a high molecular weight and shows little shearing, making it suitable for a range of downstream applications or long term storage.

### INTENDED USE

This kit is intended for research use only for the extraction of DNA from plant tissue and filamentous fungi.

### CALCULATION OF CENTRIFUGAL FORCE

To ensure that the Nucleon protocols are universally applicable to all centrifuges, centrifugal force is expressed in g-force rather than r.p.m values. To convert from r.p.m. to g, please refer to the rotor manufacturers manual. If this is not available please use the formula illustrated below.

$$g = 1.12r \left( \frac{\text{r.p.m}}{1000} \right)^2$$

$$\text{r.p.m} = 1000 \sqrt{\frac{g}{(1.12r)}}$$

r = maximum radius of rotor in mm

3. *Brown DNA pellet due to phenol/tannin mediated oxidation*

The PhytoPure protocol has been tested with a wide range of plant materials in which mercaptoethanol is not required for oxidation-free DNA extraction. However, if very high levels of phenol/tannins are anticipated in the plant material the addition of 2-mercaptoethanol to Reagent 1 to a final concentration of 10mM may alleviate this problem.

4. *The DNA pellet will not re-dissolve or re-dissolves only slowly*

This occurs if the DNA pellet is over dried. Please follow the recommended drying conditions. To enhance the dissolution process the samples can be incubated at 65°C for 2-3 hours after addition of the re-suspension solution.

### KIT CONTENTS

	SL-8510	SL-8511	Number of Bottles	Storage Conditions
Reagent 1	31mL	245mL	1	15-25°C
Reagent 2	11mL	85mL	1	15-25°C
PhytoPure Resin	6mL	12mL	1	15-25°C
Protocol Booklet	-	-	N/A	-

### Additional materials required but not supplied:

Dry ice or liquid nitrogen, chloroform, polypropylene centrifuge tubes, ethanol, mercaptoethanol (optional), propan-2-ol, RNase (optional).

### SAFETY

The Nucleon extraction kit components Reagents 1 and 2 contain chemical reagents that may be hazardous. These chemicals are irritating to eyes, respiratory system and skin. They may be harmful by inhalation, ingestion or skin absorption. Wear gloves and eye/face protection. In case of skin contact wash affected area with copious amounts of soap and running water. In case of eye contact wash out with water or saline for at least 15 minutes. If the chemical has been confined to the mouth, give large quantities of water as a mouthwash. Do not swallow. In case of ingestion, give 250mL of water to dilute. Seek medical advice.

### PREPARATION FOR USE

On receipt of kit check contents against the above list.

### STORAGE/STABILITY

PhytoPure kits are stable at the storage temperatures indicated above for 36 months (3 months once opened) if handled under aseptic conditions.

## PROTOCOLS

Please read the following before use.

- **All reagents are added at ambient temperature unless otherwise stated and refer to fresh weight of plant tissue.**
- **If dried tissue is used see Helpful Hint 1**

### PROTOCOL 1 (Product No. SL8510) For 50 x 0.1g preps from plant tissue

#### Tissue preparation

Carry out steps 1-3 as rapidly as possible.

1. Add liquid nitrogen or 3 volumes of dry ice to 0.1g (fresh weight) of plant tissue which has been frozen at  $-20^{\circ}\text{C}$ .
2. Grind the tissue in dry ice or liquid nitrogen to yield a free-flowing powder.
3. Transfer the powder using chilled instruments to a suitable polypropylene centrifuge tube.

#### Cell lysis

1. Add 600 $\mu\text{L}$  of Reagent 1, ensuring that all the reagent ingredients are fully dissolved. Mix thoroughly.
2. Optional incubations with RNase and mercaptoethanol can be carried out at this stage.
3. Add 200 $\mu\text{L}$  of Reagent 2.
4. Invert several times until a homogeneous mixture is obtained.
5. Incubate the mixture at  $65^{\circ}\text{C}$  in a shaking water bath for 10 minutes. Alternatively, regular manual agitation during the incubation will suffice.
6. Place the sample on ice for 20 minutes.

#### Deproteinisation

1. Remove sample from ice and add 500 $\mu\text{L}$  chloroform which has been stored at  $2-8^{\circ}\text{C}$ .
2. Add 100 $\mu\text{L}$  of PhytoPure Resin (ensure the resin is fully re-suspended before use)
3. Shake at room temperature on a tilt shaker for 10 minutes. Alternatively, regular manual agitation for similar period will suffice.
4. Centrifuge at 1300g for 10 minutes.
5. Without disturbing the resin layer, transfer (using a pipette) the DNA-containing upper phase ABOVE the PhytoPure Resin layer (brown in colour) into a fresh tube.

## Possible causes post extraction:

### 1. Inaccurate absorbance measurement

Check the calibration of the spectrophotometer using a standard DNA solution.

### 2. Poor Quality of DNA

DNA quality can be assessed by the measurement of its absorbance in solution at 260nm and 280nm. The  $A_{260/280}$  ratio should be greater than 1.8. The DNA pellet prior to dissolution should be white in colour and free of carryover contaminants. Some causes for poor DNA quality are given below.

#### 1. Low ratios due to protein contamination

The chemistry could be overloaded due to the presence of too many DNA containing cells. This could result in the incomplete pelleting of the resin due to the high viscosity of the sample and inefficient protein removal. See above for chemistry overloading problems and solutions.

Protein carryover might also occur due to a loose pellet or inaccurate pipetting. Every effort should be taken to follow the protocol as carefully as possible ensuring that the g-forces used are those specified in the protocol. If your centrifuge cannot achieve the correct g-force, spin for a longer time to achieve the same effect. For example, 1000g for 10 minutes is equivalent to 2000g for 5 minutes.

The re-suspended DNA solution can be cleaned up further by the addition of PhytoPure resin to a final concentration of 1% (v/v). Mix the resuspended DNA and resin for a few minutes and recentrifuge the sample to sediment out the resin. Transfer the supernatant to a fresh tube.

#### 2. PhytoPure resin carry over into the DNA pellet

It is possible for some PhytoPure resin to be carried over into the DNA pellet, which then appears brown/red in colour. This is unlikely to happen if the protocol is carefully followed and the correct centrifugation speeds are used (refer to the formulae below). In the event of carryover, the PhytoPure resin has been demonstrated not to interfere with downstream processes such as PCR<sup>†</sup>, however, if removal is required, a brief centrifugation at 3000g should pellet any resin present.

## TROUBLESHOOTING

### 1. Low Yield of DNA

#### Possible causes at the Tissue Lysis Stage:

##### 1. *Too few nucleated cells present in the starting sample*

The plant material needs to be accurately weighed ( $\pm 10\%$ ) and of reasonable quality. Check the sample mass prior to cell lysis step. If a low yield is expected due to extremely small sample mass, adding a carrier DNA (e.g. denatured herring sperm DNA) or glycogen (1 $\mu$ L of 20mg/mL glycogen) with the propan-2-ol at the DNA precipitation stage may enhance recovery.

##### 2. *Too many nucleated cells present in the starting sample*

This may result in incomplete lysis due to overloading of the chemistry. Check the amount of plant material being extracted does not exceed that recommended. The ratio of reagent volumes to tissue is important. Do not exceed the stated amounts of tissue. Either reduce the amount of starting material or increase reagent volumes proportionally.

A sample that overloads a chemistry can be re-extracted with chloroform and PhytoPure resin as per the protocol. This will remove large amounts of cell debris prior to the addition of PhytoPure resin, which would otherwise become entrapped in the debris preventing polysaccharide binding.

Alternatively, centrifuge the sample at greater than 1300g after PhytoPure resin addition and subsequent shaking as per protocol. This will cause PhytoPure resin to spin to the bottom of the tube rather than form a barrier at the interface. Care must therefore be taken when removing the aqueous upper phase.

##### 3. *Incomplete tissue lysis*

Incomplete cell lysis can be due to the detergent in Reagent 1 coming out of solution. The detergent can be re-dissolved by heating Reagent 1 to 37°C for 10 minutes prior to use. Furthermore, lysis of the sample can be improved by ensuring the plant material is fully ground to a free-flowing powder.

## DNA precipitation

1. Add an equal volume, approximately 600-800 $\mu$ L of cold propan-2-ol.
2. Gently invert the tube until DNA precipitates.

## DNA washing

1. Centrifuge at a minimum of 4000g for 5 minutes to pellet the DNA.
2. Wash the DNA pellet with 1mL 70% ethanol.
3. Centrifuge at a minimum of 4000g for 5 minutes to pellet the DNA.
4. Discard the supernatant.
5. Air-dry the DNA pellet for 10 minutes. Do not exceed this time as this may render the DNA difficult to dissolve.
6. Carefully remove remaining ethanol droplets from the tube and resuspend the DNA in TE buffer or sterile water as required (e.g. 100-200 $\mu$ L )

**PROTOCOL 2** (Product No. SL8511)  
For 50 x 1.0g preps from plant tissue

**Tissue preparation**

Carry out steps 1-3 as rapidly as possible

1. Add liquid nitrogen or 3 volumes of dry ice to 1.0 g (fresh weight) of plant tissue which has been frozen at  $-20^{\circ}\text{C}$
2. Grind the tissue in dry ice or liquid nitrogen to yield a free-flowing powder.
3. Transfer the powder using chilled instruments to a suitable polypropylene centrifuge tube.

**Cell lysis**

1. Add 4.6mL of Reagent 1 ensuring that all the reagent ingredients are fully dissolved. Mix thoroughly.
2. Optional incubations with RNase and mercaptoethanol can be carried out at this stage.
3. Add 1.5mL of Reagent 2.
4. Invert several times until a homogeneous mixture is obtained.
5. Incubate the mixture at  $65^{\circ}\text{C}$  in a shaking water bath for 10 minutes. Alternatively, regular manual agitation during the incubation will suffice.
6. Place the sample on ice for 20 minutes.

**Deproteinisation**

1. Remove sample from ice and add 2mL chloroform which has been stored at  $-20^{\circ}\text{C}$ .
2. Add 200 $\mu\text{L}$  of PhytoPure Resin (ensure the resin is fully re-suspended before use).
3. Shake at room temperature on a tilt shaker for 10 minutes. Alternatively, regular manual agitation for a similar period will suffice.
4. Centrifuge at 1300g for 10 minutes.
5. Without disturbing the PhytoPure Resin layer, transfer (using a pipette) the DNA-containing upper phase ABOVE the PhytoPure resin layer (brown in colour) into a fresh tube.

**DNA precipitation**

1. Add an equal volume of cold propan-2-ol.
2. Gently invert the tube until DNA precipitates.

**DNA washing**

1. Centrifuge at 4000g for 5 minutes to pellet the DNA
2. Wash the DNA pellet with at least 1mL 70% ethanol
3. Centrifuge at 4000g for 5 minutes to pellet the DNA
4. Discard the supernatant
5. Dry the DNA pellet under vacuum or air dry for 10 minutes. Do not exceed this time as this may render the DNA difficult to dissolve.

6. Resuspend the DNA in TE buffer or water as required.

**HELPFUL HINTS**

**Note 1:**

Where dried tissue is used the amount of material to be extracted should be reduced by a factor of five

**Note 2:**

To extract larger amounts, the volumes in the protocols can be scaled-up proportionately to the increased weight of tissue. However the PhytoPure resin should be scaled up according to **tube size** i.e. 1.5mL: 100 $\mu\text{L}$ ; 5mL: 200 $\mu\text{L}$ ; 10mL: 300 $\mu\text{L}$ .

**Note 3:**

The extracted DNA may contain small amounts of RNA. Should RNA-free DNA be required, an RNase digestion step can be included either at the end of the protocol or by adding RNase to a final concentration of 20 $\mu\text{g}/\text{mL}$  after the addition of Reagent 1 followed by incubation at  $37^{\circ}\text{C}$  for 30 minutes. However, this has been found to be unnecessary in most cases.

**Note 4:**

It is important to ensure that the resin bottle contains equal proportions of resin to buffer. Allow the resin to settle and add sterile water if necessary.

**Note 5:**

Precipitated DNA can be hooked out using a heat-sealed Pasteur pipette. A 70% ethanol wash is not necessary and the DNA should be placed directly into TE or sterile water.