GE Healthcare

illustra Nucleon Phytopure Genomic DNA Extraction Kits

Product Booklet

Codes: RPN8510
RPN8511

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1. Components of the system

illustra™ Nucleon PhytoPure, plant and fungal DNA extraction kits (RPN8510 / 8511) consist of:

	Product Code	
	0.1 g samples RPN8510	**1.0 g samples RPN8511**
Component		
Reagent 1	31 ml	245 ml
Reagent 2	11 ml	85 ml
Phytopure Resin	6 ml	12 ml

2. Safety warnings and precautions

Warning: For research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Note that the protocol requires the use of:
Chloroform: carcinogen, Cat 3, harmful, irritant.
Mercaptoethanol: toxic.
Isopropanol: flammable.
Ethanol: highly flammable.

Please follow the manufacturer's Safety Data Sheet relating to the safe handling and use of these reagents.

3. Storage and stability

Storage
Store at room temperature.

Stability
Nucleon kit components are stable for up to 18 months, 3 months once opened, when stored under the recommended conditions. Performance is consistent when stored under the recommended conditions using the recommended procedures.

Expiry
For expiry date please refer to outer packaging label.

4. Description

Nucleon™ PhytoPure™ systems from GE Healthcare for extracting DNA from plant and fungal samples are 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 is also considerably simpler.

While 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 the further enzymatic analysis of the DNA.

Nucleon Phytopure DNA extraction systems have been developed specifically to solve these problems. The system protocol is rapid and eliminates the requirement for phenol or cetyltrimethylammonium bromide (CTAB). After breaking of the cell wall, the cells are lysed in a reagent containing potassium SDS which is known to form complexes with proteins and polysaccharides. Chloroform is then added along with the specially modified Nucleon PhytoPure proprietary resin. This resin covalently binds polysaccharides, resulting in a high quality DNA preparation at the end of the protocol.

Role of Nucleon PhytoPure resin in plant DNA extraction.

The specially modified Nucleon PhytoPure resin suspension performs two vital functions during the extraction procedure.
Nucleon PhytoPure resin particles contain free boric acid (-B(OH)2) groups. This resin is reactive towards polysaccharides through a well established mechanism whereby the boric acid reacts with 1,2

dihydroxy compounds to yield a cyclic boric acid ester.
The polysaccharides are bound by Nucleon PhytoPure resin particles and therefore removed from the sample.
Nucleon PhytoPure resin forms a semi-solid stratum between a lower organic and upper DNA-containing aqueous phase during the extraction process. This facilitates DNA recovery without repeated partitions, ensuring high yields of high quality DNA suitable for further analysis by restriction enzyme digestions, RAPD and AFLP.

Plant and Filamentous Fungi species from which DNA has been successfully extracted using PhytoPure.

Arabidopsis	*Helianthus annus*	*Pisum sativum*
Araucaria araucaria	*Helianthus tuberosus*	*Rhododendron spp*
Beta vulgaris	*Hevea braziliensis*	*Salix spp*
Brassica oleracea	*Humulus lupulus*	*Solanum tuberosum*
Brassica napus	*Irvingia gabonensis*	*Sorghum seeds*
Capsicum annuum	*Lolium*	*Sphagnum spp*
Capsicum frutescens	*Lotus japonicus*	*Spinacea oleracea*
Cereals *(barley, maize, rye, wheat)*	*Lupins albus*	*Swietenia macrophyla*
Cocos nucifera	*Lycopersicon esculentum*	
Cryptomeria	*Malus spp*	
Eucalyptus globulis/grandis	*Musa spp*	*Aspergillus niger*
Fragaria x ananassa	*Nicotiana*	*Mortierella alpina*
Fucus	*Phaseolus vulgaris*	*Colletotrichum gloeosporioides*
Gossypium hirsutum	*Pinus sylvestris*	*Septoria nodorum*

5. Principle steps in PhytoPure extraction

breaking of cell wall

cell lysis with potassium/SDS

DNA extraction with Nucleon PhytoPure resin and chloroform

DNA precipitation

DNA washing

6. Critical parameters

- Carry out steps 1–3 as quickly as possible.
- Use chloroform stored at -20°C as this has been found to increase the efficiency of removal of complexed proteins/polysaccharides.
- Spin speed of 1300 g allows the resin to form a barrier at the organic/aqueous interface, facilitating the removal of the upper phase without danger of contamination.
  Using a higher spin speed may cause the resin to spin to the bottom of the tube, and more care must be taken in removing the upper phase.

7. Additional equipment and solutions required

7.1 Equipment

- Microcentrifuge
- Bench top centrifuge
- Assorted range of high precision pipettes
- Pasteur pipettes
- Polypropylene centrifuge tubes
- Water bath
- Rotary mixer

7.2 Solutions

- Chloroform, AnalaR™ grade or similar (stored at -20°C)
- Isopropanol, AnalaR grade or similar
- Ethanol, AnalaR grade or similar
- RNase (if required)
- Mercaptoethanol (if required)
- Liquid nitrogen / dry ice

8. Protocols

8.1 illustra Nucleon PhytoPure for small samples, 0.1 g (RPN8510)

The protocol requires the use of cold isopropanol, 70% ethanol and chloroform stored at -20°C.

Protocol	Notes
Breaking of the cell wall	
1. Add three volumes of dry ice (or liquid nitrogen if preferred) to 0.1 g (fresh weight) of plant tissue which has been frozen at -20°C.	**1.** The volumes presented in the protocol are for fresh weight of tissue. If dried tissue is to be used, please reduce the weight of tissue to be extracted by approximately a factor of 5. If the user requires to extract larger amounts of tissue, the volumes in the protocol can be scaled-up proportionately to the increased weight of tissue. However, PhytoPure resin should be scaled up according to tube-size ie:
2. Grind the tissue in the dry ice (or liquid nitrogen) to yield a free flowing powder.	

tube size (ml)	vol. of resin (µl)
1.5	100
5	200
10	300

Protocol	Notes
3. Transfer the powder, using a chilled spatula, to a suitable polypropylene centrifuge tube.	
Cell Lysis	
4. Add 600 µl of Reagent 1, ensuring that all the reagent ingredients are fully dissolved. (Optional incubations with mercaptoethanol and RNase should be performed at this stage, see note 4).	**4.** This protocol has been found not to require mercaptoethanol as a constituent. If the user prefers to include this reagent, add it at this stage, to a concentration in Reagent 1 of 10 mM. The extracted DNA may contain small amounts of RNA. Should RNA-free DNA be required, perform an RNase digestion at this step by adding RNase to a concentration of 20 µg/ml after the addition of Reagent 1. Incubate at 37°C for 30 minutes.
5. Mix thoroughly with a spatula.	
6. Add 200 µl of Reagent 2.	
7. Invert several times until a homogeneous mixture is obtained.	
8. Incubate at 65°C in a shaking	

Protocol	Notes
water bath for 10 minutes. Alternatively, regular manual agitation during the incubation is sufficient.	
9. Place sample on ice for 20 minutes.	
DNA extraction	
10. Remove sample from ice and add 500 µl of chloroform which has been stored at -20°C.	**10.** Chloroform at -20°C is most effective in aiding the removal of complexed proteins/polysaccharides.
11. Add 100 µl of Nucleon PhytoPure DNA extraction resin suspension.	**11.** Ensure the resin is fully suspended by vigorous shaking immediately before use. It is important to ensure that the resin bottle contains equal proportions of resin to buffer. Allow the resin to settle and add sterile TE buffer if necessary.
12. Shake on a tilt shaker for 10 minutes, at room temperature. Alternatively, regular manual agitation during the incubation is sufficient.	
13. Centrifuge at 1300 g for 10 minutes.	

Protocol	Notes
14. Without disturbing the Nucleon resin suspension layer, transfer (using a pipette) the upper DNA containing phase, above the brown resin layer, into a fresh tube.	**14.** The upper phase may appear green and cloudy but does not affect the quality of the DNA. An additional spin at speeds greater that 1300 g may be used to clarify the transferred aqueous phase.
DNA precipitation	
15. Add an equal volume of cold isopropanol.	
16. Gently invert the tube until DNA precipitates.	**16.** Precipitated DNA may be hooked out at this stage, using a heat-sealed pipette. This DNA does not require a 70% ethanol wash and should be placed directly into TE buffer or sterile water.
17. Centrifuge at a minimum of 4000 g for 5 minutes to pellet the DNA.	
18. Wash the DNA pellet with cold 70% ethanol.	
19. Centrifuge at a minimum of 4000 g for 5 minutes to pellet the DNA.	
20. Discard the supernatant.	

Protocol	Notes
20. Discard the supernatant.	
21. Air-dry the DNA pellet for 10 minutes. Remove any remaining ethanol droplets from the tube.	
22. Resuspend the DNA in TE buffer or water as required.	**22.** An RNase digestion can be performed at this stage if required (see note 4).

8.2 illustra Nucleon PhytoPure for large samples, 1.0g (RPN8511)

The protocol requires the use of cold isopropanol, 70% ethanol and chloroform stored at -20˚C.

Protocol	Notes
Breaking of the cell wall	
1. Add three volumes of dry ice (or liquid nitrogen if preferred) to 1.0 g (fresh weight) of plant tissue which has been frozen at -20˚C.	**1.** The volumes presented in the protocol are for fresh weight of tissue. If dried tissue is to be used, please reduce the weight of tissue to be extracted by approximately a factor of 5. If the user requires to extract larger amounts of tissue, the volumes in the protocol can be scaled-up proportionately to the increased weight of tissue. However, PhytoPure resin should be scaled up according to tube-size ie:
2. Grind the tissue in the dry ice (or liquid nitrogen) to yield a free flowing powder.	
3. Transfer the powder, using a chilled spatula, to a suitable	

tube size (ml)	vol. of resin (µl)
1.5	100
5	200
10	300

Protocol	Notes
polypropylene centrifuge tube.	
Cell Lysis	
4. Add 4.6 ml of Reagent 1, ensuring that all the reagent ingredients are fully dissolved. (Optional incubations with mercaptoethanol and RNase should be performed at this stage, see note 4)	**4.** This protocol has been found not to require mercaptoethanol as a constituent. If the user prefers to include this reagent, add it at this stage, to a concentration in Reagent 1 of 10 mM. The extracted DNA may contain small amounts of RNA. Should RNA-free DNA be required, perform an RNase digestion at this step by adding RNase to a concentration of 20 µg/ml after the addition of Reagent 1. Incubate at 37˚C for 30 minutes.
5. Mix thoroughly with a spatula.	
6. Add 1.5 ml of Reagent 2.	
7. Invert several times until a homogeneous mixture is obtained.	
8. Incubate at 65°C in a shaking water bath for 10 minutes. Alternatively, regular manual agitation during the incubation is sufficient.	
9. Place sample on ice for 20	

Protocol	Notes
minutes.	
DNA extraction	
10. Remove sample from ice and add 2 ml of chloroform which has been stored at -20°C.	**10.** Chloroform at -20°C is most effective to aid the removal of complexed proteins/polysaccharides.
11. Add 200 µl of Nucleon PhytoPure DNA extraction resin suspension.	**11.** Ensure the resin is fully suspended by vigorous shaking immediately before use. It is important to ensure that the resin bottle contains equal proportions of resin to buffer. Allow the resin to settle and add sterile TE buffer if necessary.
12. Shake on a tilt shaker for 10 minutes, at room temperature. Alternatively, regular manual agitation during the incubation is sufficient.	
13. Centrifuge at 1300 g for 10 minutes.	
14. Without disturbing the Nucleon resin suspension layer, transfer (using a pipette) the upper DNA containing phase, above the brown resin layer, into a fresh tube.	**14.** The upper phase may appear green and cloudy but does not affect the quality of the DNA. An additional spin at speeds greater that 1300 g may be used to clarify the transferred

Protocol	Notes
	aqueous phase.
DNA precipitation	
15. Add an equal volume of cold isopropanol.	
16. Gently invert the tube until DNA precipitates.	**16.** Precipitated DNA may be hooked out at this stage, using a heat-sealed pipette. This DNA does not require a 70% ethanol wash and should be placed directly into TE buffer or sterile water.
17. Centrifuge at a minimum of 4000 g for 5 minutes to pellet the DNA.	
18. Wash the DNA pellet with cold 70% ethanol.	
19. Centrifuge at a minimum of 4000 g for 5 minutes to pellet the DNA.	
20. Discard the supernatant.	
21. Air-dry the DNA pellet for 10 minutes. Remove any remaining ethanol droplets from the tube.	
22. Resuspend the DNA in TE buffer or water as required.	**22.** An RNase digestion can be performed at this stage if required (see note 4).

9. Trouble shooting guide

Problem	Possible cause	Remedy
1. Extracted DNA fails to amplify by PCR or digest with restriction enzymes.	**1.** The DNA is impure requiring modifications of the procedure to reduce the contamination.	**1.1** Ensure that the plant tissue is fully ground at the start of the protocol.
		1.2 Ensure full resuspension of the ground material in Reagent 1 and that Reagent 2 is thoroughly mixed with the lysate. FULLY dissolved material is essential for maximum yield and purity.
2. On isopropanol precipitation, a large pellet forms which is obviously not comprised of DNA alone.	**2.** The DNA is impure requiring modifications of the procedure to reduce the contamination.	**2.** Check the amount of plant material being extracted does not exceed that recommended for each protocol. The ratio of reagent volumes to tissue is

Problem	Possible cause	Remedy
		important. Do not exceed the stated amounts of tissue. Either reduce the amount of starting material or increase reagent volumes proportionately.
3. On resuspension of the DNA pellet in water or TE buffer the resulting solution is viscous and perhaps cloudy.	**3.** The DNA is impure requiring modification of the procedure to reduce the contamination.	**3.** A post extraction clean-up on the resuspended DNA solution at the end of the protocol may be performed. Add PhytoPure resin to a final concentration of 1%, mix for a few minutes and spin out the PhytoPure resin.
4. Large amounts of cellular debris present.	**4.** The system may be overloaded or complete lysis has not occurred.	**4.1** See the solutions above relating to reagent mixing and amount of tissue being extracted.
		4.2 Perform an additional

Problem	Possible cause	Remedy
		chloroform extraction after cooling on ice for 20 minutes. Centrifuge at 1300 g for 10 minutes and retain the aqueous upper phase. Re-extract 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.
		4.3 Centrifuge at greater than 1300 g after PhytoPure resin addition and subsequent shaking as per

Problem	Possible cause	Remedy
		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.
5. DNA pellet appears brown.	**5.** Phenol / tannin mediated oxidation occured.	**5.** In the wide variety of plant materials tested mercaptoethanol has not been found to be necessary. However, in plant tissues with very high levels of phenol / tannins the addition of 2-mercaptoethanol to the lysis buffers (Reagent 1 & 2 combined) to a final concentration of 10 mM may alleviate this

Problem	Possible cause	Remedy
		problem.

10. Additional information

10.1 Calculation of centrifugal force

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

$g = 1.12r\ (rpm/1000)^2$

$rpm = 1000\sqrt{(g/1.12r)}$

r = maximum radius of the rotor in mm

11. DNA extraction products

illustra Nucleon HT,
for hard tissue, and paraffin sections,
50 preparations of up to 25 mg per prep. **RPN8509**

illustra Nucleon PhytoPure,
for plant and fungal DNA extraction kit,
50 preparations of 0.1 g. **RPN8510**

illustra Nucleon PhytoPure,
for plant and fungal DNA extraction kit,
50 preparations of 1.0 g. **RPN8511**

illustra Nucleon BACC1,
for 50 preparations of 1 ml whole blood
or cultured cells (1 to 3 x 10^6). **RPN8501**

illustra Nucleon BACC2,
for 50 preparations of 10 ml of whole blood
or cultured cells (3 x 10^6 to 1 x 10^7). **RPN8502**

illustra Nucleon BACC3,
for 50 preparations of 10 ml of whole blood. **RPN8512**

12. Legal Section

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RPN8510PL Rev B 11/2007