

WARRANTY

Nucleplex Plant DNA Extraction Kits come with a 'no quibble' warranty. We trust that this Nucleplex 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 Nucleoplex range:

33100	Nucleoplex Plasmid DNA extraction kit for up to 192 extractions
33110	Nucleoplex Plasmid High Usage Kit for up to 1920 extractions (plastic ware not included)
33200	Nucleoplex BAC DNA extraction kit for up to 192 extractions
33210	Nucleoplex BAC High Usage Kit for up to 1920 extractions (plastic ware not included)
33310	Nucleoplex Plant High Usage Kit for up to 1920 extractions (plastic ware not included)

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
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
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
SL8512	Nucleon BACC3 kit for 50 extractions of up to 10mL of whole blood or cell cultures
44100	Non-chloroform Blood kit for 50 preps of 10mL whole blood
44200	Non-chloroform Mouse Tail kit for 50 preps of 1cm mouse tail
44201	Non-chloroform Mouse Tail kit for 200 preps of 1cm mouse tail
44300	Non-chloroform Plant kit for 50 extractions of 0.1g of plant tissue

Please contact your local supplier for further information.

Nucleoplex™ is a trademark of Tepnel Life Sciences PLC.

Nucleon® and PhytoPure® are registered trademarks of Tepnel Life Sciences PLC.

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

BigDye™ is a trademark of Applied BioSystems.

NUCLEOPLEX™ PLANT DNA KIT

Product No. 33300

(4 x 96 preps)

NB: Please refer to kit contents section for storage instructions



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INTENDED USE:

This kit is for research use only and is to be used solely for the intended purpose of extracting DNA from Plant tissues.

Notes:**KIT CONTENTS:**

Kit Components	Number of Items	Storage Temp	Component Number
Plant Lysis Buffer (A) ~95mL	2 x 125mL	15-25 °C	33300/02
Plant Wash Buffer (B) ~98mL	4 x 250mL	15-25 °C	33300/05
Plant Neutralisation Buffer (C) ~110mL	2 x 125mL	15-25 °C	33300/03
Plant Elution Buffer (D) ~170mL	2 x 250mL	15-25 °C	33300/01
Container for the preparation of Magnetic Particle Suspension	2 x 60mL	15-25 °C	30013
Plant Magnetic Particles ~2mL (each tube)	4 x 2mL	15-25 °C	33300/04
RNase A Solution ~0.4mL	2 x 60mL	-20 °C	33300/06
Proteinase K	2 x 0.5mL	-20 °C	33300/07
Microtitre Elution Plate	4	N/A	P0093
Plate Sealing Membranes	4	N/A	30011
Columns (Racked)	4 x 96	N/A	R0013
Filter Tips (Racked)	4 x 96	N/A	30020
96 Tubes (Racked)	4 x 96	N/A	30012
Deep Well Plate	4	N/A	P0080
Tube caps (strips of 8)	2 x 125	N/A	C0070

TROUBLESHOOTING & HELP

- **The sample tube rack will not fit into the instrument.** The base of the rack may become displaced during grinding or centrifugation which will prevent the rack fitting into the instrument. Gently push the base back up into place. Alternatively, a rack that has not been cooled after incubation will have expanded, making it difficult to place it correctly into position.
- **How often do I need to change the reagent bottles?** The lysis, neutralisation and elution reagent bottles (A, C & D) contain sufficient reagents for 2 x 96 preps or 4 x 48 preps. The wash bottles (B), once made up with ethanol, only contain enough reagents for 1 x 96 or 2 x 48 preps and will therefore require changing more often than the other bottles. It is good practice to empty the waste bottle (and the waste bin) at the end of every run.
- **The wash reagent bottle still appears to be quite full after a run.** The extended wash protocol uses more wash solution (65% ethanol) than the plant protocol. It is therefore possible that after using the plant protocol, the wash bottle appears to be quite full.
- **I have beads in my eluant.** It is not unusual to find beads in the eluant when using the plant protocol. They will not bind the DNA upon storage and can easily be avoided when pipetting (they do not interfere with downstream reactions if transferred). The extended plant protocol is less likely to give rise to beads in the eluant.
- **Can the instrument ever crash?** Yes but this is easily avoided.
 - Ensure all the plastic ware has been fitted correctly in the correct positions. Pay particular attention that all spent column and filter racks have been removed before loading new racks. Also ensure that the filter tip racks have been pushed down into the retaining clips on the filter loading block. Bend the retaining clips back out if they become deformed with constant use. Ensure that the column rack is pushed down onto the loading block.
 - Do not overload the input tubes. If using coarse material use less than 50mg as the filter tips may not be able to be inserted sufficiently into this material during filtration causing the filters, in extreme cases, to protrude into the path of the X axis.

See www.tepnel.co.uk for additional literature and information

ITEMS REQUIRED BUT NOT SUPPLIED:

- 4 x 183mL analytical grade ethanol (greater than 96%) is required to make up the Plant Wash Buffer.
- 4 x 38mL analytical grade isopropanol (greater than 96%) is required to make up the Magnetic Particle Suspension.
- 2 x 39.6mL analytical grade isopropanol (greater than 96%) is required to make up the RNase Suspension.
- A centrifuge and rotor capable of spinning deep well plates at 1500 x g
- A “Bead beater” or “Mixer mill” capable of disrupting samples in a 96 sample, deep well plate
- A 65°C oven or (water bath)

NOTES:

- A Dual Trough is required to contain the RNase and Magnetic Particles. This is not supplied in the kit. A pack of 5 is supplied with the instrument. Additional packs of 5 (Product No. 33001) are available from your local supplier.
- The kit contains sufficient materials and reagents for the preparation of 4 x 96 samples. If multiple extractions of less than 48 samples are performed, additional reagents will be required. Please contact your local supplier for more information.
- Lysis Buffer may form a precipitate when stored at temperatures below 15°C . Re-dissolve before use by heating to 30°C for 5 minutes.

PRECAUTIONS:

- Plant Lysis Buffer contains sodium dodecyl sulphate (SDS) which may be hazardous. In case of contact with skin, wash the contaminated area with large amounts of water.
- Plant Neutralisation Buffer contains guanidine isothiocyanate which may be hazardous. In case of contact with skin, wash the contaminated area with large amounts of water.
- Refer to Material Safety Data Sheets for SDS and guanidine isothiocyanate for specific details on safety issues with these chemicals.

PREPARATION FOR USE:

- Check the kit contents against the list on page 2 and contact your supplier if there are any discrepancies.
- Either fill to the beginning of the bottle neck or add 183mL of (96%) ethanol to each Plant Wash Buffer (B) bottle. Tick the box on the label when added.
- Either fill to the level marked on the label or add 38mL of (96%) isopropanol to the container for the preparation of the Magnetic Particle Suspension. Tick the box when added.
- Ensure the Magnetic Particles in the 2mL tubes are completely resuspended by vortexing thoroughly. Pipette all the contents of one tube containing the Magnetic Particles into a bottle for the Magnetic Particle Suspension (if required, rinse the tube with isopropanol from the 38mL of isopropanol required to prepare the Magnetic Particle Resuspension). Replace the lid of the 60mL bottle and shake the combined contents well to ensure thorough resuspension of the Magnetic Particles. The Magnetic Particles will settle over time, therefore the suspension should always be thoroughly mixed by vortexing or shaking before use. The Magnetic Particle Resuspension has a shelf life of 3 months at 15-25°C.
- **Please note** that two empty 60mL bottles are supplied for the preparation of the Magnetic Particle Suspension (vol. 40mL each when made up), along with four tubes of 2mL of Magnetic Particles. Once prepared, 40mL of Magnetic Particle Suspension is sufficient to process 1 x 96 or 2 x 48 samples. Bottles should be retained for the preparation of subsequent 40mL volumes of Magnetic Particle Suspension.
- Either fill to the level marked on the label or add 39mLs of (96%) isopropanol to the containers for the preparation of the RNase A Suspension.
- Add the contents of a vial of proteinase K to a bottle of lysis solution. Swirl the bottle gently to ensure the powder dissolves.
- **Once opened, and made up, Reagents A-D have a shelf life of 3 months at 15-25°C.**

TROUBLESHOOTING & HELP

- **How do I calculate the concentration of my DNA?** Do not use quantitation by OD. Although this may be recommended by the manufacturers of other kits such calculations are likely to be wildly misleading. The Nucleplex chemistry contains reagents which enhance DNA purity and prevent this common but flawed method being used (although 260:280 ratios and 260:230 ratios can still be used to indicate DNA quality). Densitometry following agarose gel electrophoresis on a 1% gel is reliable if intact DNA is obtained. DNA specific dyes, such as picogreen, offer a gold standard method.
- **My yield is less than expected.** Consider the following:
 - Lysis and disruption are critical in obtaining high yield (see p.8). Make sure that the plant tissue has been completely disrupted (no visible signs of tissue structure) - extend the period of grinding if necessary.
 - If processing long leaf sections (such as lavender), cut the leaves into ~3mm sections. Long, vertical leaf sections will sit straight in the input tubes and the ball bearings will not impact on the tissues, leading to very low yield.
 - The enzyme which is present in the lysis solution will lose activity over time. A three month shelf life is assigned to the lysis solution once the enzyme has been added.
- **My downstream application has failed.** If your experimental controls indicate that the DNA is the root cause of the failure consider the following.
 - Has the DNA been diluted within the required range for the downstream application?
 - Has quantification been made using a reliable method?
 - Repeat the preparation using less material
 - Try using the extended plant protocol
 - Try using an alternative lysis protocol

TROUBLESHOOTING & HELP

- **Which lysis protocol should I use?** The standard lysis protocol is suitable for most sample types. The other lysis protocols have different purification characteristics (see p. 6 & 7) which may be useful if the standard lysis protocol is not suitable for your particular requirements.
- **Which main protocol should I use?** The main protocols are used to process the samples after lysis/disruption and off-line incubation, cooling and centrifugation. The extended plant protocol may be useful for difficult sample types (2.5 hours for 96 samples) but the plant protocol (1.75 hours for 96 samples) is suitable for most tissues (see p.9).
- **NOTE:** It may be worth testing all the common plant tissues used in your laboratory with all combinations of the lysis and main protocols when first using the instrument.
- **What advice can you give on processing difficult sample types?** If your samples are known to present difficulties you should consider the following
 - The Nucleplex chemistry is robust but can be subject to overloading if tissues have unusually high levels of certain substances. With this in mind it may pay to load less than 50mg of wet material (or 10mg of dry material).
 - The extended plant protocol utilises four aliquots of wash solution and may help in increasing the quality of DNA extracted from difficult plant tissues.
 - Do not incubate at 50 °C overnight. Use 1 hour or less at 65 °C.
 - Fresh, young tissues generally yield higher quality DNA than older tissues that have been removed and stored.
 - Particularly tough leaf materials that may require grinding after exposure to liquid nitrogen described in other purification processes can be broken down using the standard or low MW lysis protocols. Do not expose the input tubes to liquid nitrogen. It is not necessary and will result in split tubes.
 - With particularly coarse tissues such as pine needles, ensure that the tissues have been thoroughly disrupted before further processing. Extend the grinding if required. Load less material if tissue structure remains visible - **NOTE: OVERLOADING OR LOADING OF MATERIAL THAT HAS NOT BEEN THOROUGHLY DISRUPTED MAY, IN EXTREME CASES, CAUSE THE FILTERS TO PROTRUDE FROM THE INPUT TUBES CAUSING A MACHINE CRASH WITH THE UPPER X AXIS.**

OVERVIEW:

Plant preparation using the Nucleplex system can be broken down into four stages:

Stage 1: Tissue harvesting.

Racked tubes are supplied for harvesting your plant material. Either wet, dry, fresh or frozen tissue can be used. Caps are provided to seal the tubes and must be removed before the lysis protocol. Do not 'flash-freeze' by exposure to liquid nitrogen.

Stage 2: Tissue disruption.

The racked tubes are placed on the instrument to receive lysis solution. The tubes are then sealed with caps and the tissues disrupted using a mixer mill (also known as a bead beater or grinder). A short period of centrifugation may be required after milling to remove material from the caps.

Stage 3: Off-line Incubation.

The disrupted tissues are incubated off-line at an elevated temperature and then cooled. Prior to loading on the instrument, the samples are subjected to a short period of centrifugation. If more than one rack is subjected to disruption, the racks may be stored at 4 °C (for a maximum of 12 hours) after centrifugation before further processing.

Stage 4: DNA Purification

The samples are placed onto the instrument for processing using a "walk away" automated protocol. The DNA is eluted in 10mM Tris for immediate use or storage.

PROCESS INFORMATION

TISSUE DIVERSITY

The plant kingdom is diverse and different tissues (i.e. root, leaf, stem, etc.) differ in their characteristics. Tissue characteristics may also vary with the season or other environmental conditions. Bearing this variation in mind, we advise that 50mg of wet tissue or 10mg of dry tissue is used (these can be either fresh or frozen) for the majority of plant tissues. The standard lysis protocol, in combination with the main plant protocol, provides a robust method of purification for the majority of samples using these weights (see section on milling/disruption p.8, for limitations on sample input). Severe overloading will compromise the quality of the output and may compromise the automated process (see notes on milling/disruption, p.8).

HARVESTING

Tissue samples should be inserted into the sample input tubes (8 x 12 racked format containing ball bearings), such that they are pushed towards the bottom of the tube (a pipette tip is a useful tool to aid insertion, alternatively centrifugation may be used to drive some tissues to the bottom of the tubes). This is necessary as lysis solution is added by the instrument to each tube and can overflow if a plug of plant material is present at the top of the tube. Caps may be used to efficiently seal these tubes which can be frozen for storage (down to -80 °C).

SAMPLE LYSIS AND DISRUPTION

Lysis

Three lysis protocols are provided (Standard, High MW and low MW protocols) which must all be used in conjunction with a mixer mill. A "dual block version" of each protocol is available for the lysis and disruption of two sample racks and is suitable for use with mixer mills that can accommodate two sample racks. A "single block version" of each protocol is available for the lysis and disruption of a single sample rack, although a balance block will be required for centrifugation (and for some makes of mixer mill) if a single rack is processed. Each of the protocols has different characteristics affecting disruption, yield and quality. The standard lysis protocol is suitable for the majority of sample types.

IF IN DOUBT ABOUT WHICH PROTOCOL TO USE SELECT THE
STANDARD LYSIS PROTOCOL.

WORKFLOW:

Serial work flow: It is possible to take 2 racks of samples through the entire process (from lysis to elution) in one day using a serial work flow.

Parallel work flow: For additional convenience and to allow higher throughput it is possible to subject up to four racks of samples to lysis, disruption and off-line processing in a near parallel process before using the main automated protocols. In order to process 384 samples (4 x 96) in a normal working day it is advisable to use a dual block lysis protocol, a dual block mixer mill and the plant purification protocol (duration 1.75 hours for 96 samples). Samples should be subjected to centrifugation after disruption, incubation and cooling (see off-line processing, p.9) before storing at 4 °C prior to using the main protocol. The stored racks can then be processed using the main automated protocol one at a time in a serial process to allow up to 384 (4 x 96) samples to be prepped per day. The use of the extended plant protocol will reduce throughput as 2.5 hours is required to process 96 samples.

An overnight off-line incubation (see off-line processing, p.9). may be performed to support a different work flow. Disruption may take place at the end of the day followed by an overnight incubation, cooling and centrifugation the next morning prior to using the main automated protocol.

SUPPLIER DETAILS:

Consumable items required for use on the instrument may be obtained from either Tepnel Life Sciences or direct from the suppliers listed below.

2mL Deep Well Plates Povair Sciences Ltd

Cat. No. 219009

See www.povair-sciences.com for local distributors.

Microtitre Plates Invitrogen (Nalge-Nunc International)

Cat. No. 268152

www.nalgenunc.com

Sealing Caps Starlab GMBH

Cat. No. E 1702 8400

www.starlabs.de

Plate Sealing Membranes Invitrogen (Nalge-Nunc International)

Cat. No. 263366

www.invitrogen.com

6. ELUTION PLATE

Place a clean, empty Microtitre Elution Plate in **position 6**. Ensure that well A1 is placed in the rear right-hand corner of the input position (clearly labelled A1). The plant DNA is eluted into the Microtitre Elution Plate at the end of the run. The well position of the purified plant DNA in the Microtitre Elution Plate corresponds to the position of the input sample.

Start the protocol run: Ensure that all the doors are closed. Start the protocol by following the software prompts through to the start protocol screen, after entering the desired number of samples to be processed and the required elution volume. Throughout the run, the touch screen displays the status of the protocol in large text.

Remove samples: When the protocol is finished, a Protocol Complete screen is displayed. The Elution Plate should be removed for storage in appropriate conditions (4°C for short-term storage, -20°C or -80°C for long-term storage). Use a membrane to seal the plate prior to storage. The DNA is ready to use in most downstream applications, however dilutions may be required

Waste removal: At the end of the run remove all used plastic ware from the instrument. The racked sample tubes (containing the ball bearings and used filter tips) may be discarded. The Dual Trough and Wash Deep Well Plate may be re-used in subsequent runs. Discard any residual reagents in the Dual Trough. Ensure that empty Column and Filter Tip carriers are removed from the blocks at the end of the run. It is good practice to empty the liquid waste bottle and waste bin at the end of each run. Reagent bottles containing residual reagents may be left connected to the instrument for use in subsequent protocols. Once opened, reagents A-D have a shelf life of 3 months at 15-25°C.

Considerations for running protocols of less than 96 samples: This kit has been designed to process 384 samples in up to 8 runs. The instrument allows any number of Plant samples (up to 96) to be purified during a run. It is therefore important to refer to the instrument Set-Up screen to check that sufficient reagents are available to complete the run before starting a protocol.

Multiple runs of less than 96 samples may require re-use of the Wash Deep Well Plate.

Standard Lysis protocol: The majority of tissues can be processed using this protocol. Samples contained within tubes (with caps removed) are placed onto the instrument and lysis solution is automatically added to each sample. Caps are used to seal each tube and the racked tubes are then placed into a mixer mill. Once the tissue has been completely disrupted the racked tubes should be briefly subjected to centrifugation (2000g x 5 seconds) to remove any material from the caps. The caps can then be removed and discarded and the racks placed back on the instrument to receive an additional aliquot of reagent. At the end of the automated lysis protocol, the racked tubes should be incubated in an oven (see off-line processing p.9), briefly cooled and centrifuged before further processing on the instrument (see main automated process, p.9). The standard lysis protocol will allow high yields of high quality DNA with sufficient fragment size (typically 23kb+) to support the majority of downstream applications.

Low MW Lysis protocol: This protocol provides a more aggressive disruption than the standard lysis protocol and the resulting fragment size is lower but is suitable for short and medium range PCR.

Samples contained within tubes (with caps removed) are placed onto the instrument and lysis solution is automatically added to each sample. Caps are used to seal each tube and the racked tubes are then placed into a mixer mill. Once the tissue has been completely disrupted the racked tubes should be incubated in an oven briefly cooled and centrifuged (see off-line processing, p.9), before further processing on the instrument (see main automated process, p.9).

High MW Lysis Protocol: This lysis protocol provides a less aggressive means of disruption than the previously described protocols and will typically produce higher molecular weight DNA but less yield. Longer post-disruption incubation times are advised to compensate for the less efficient mechanical disruption.

Samples contained within tubes (with caps removed) are placed onto the instrument and lysis solution is automatically added to each sample. Caps are used to seal each tube and the racked tubes are then placed into a mixer mill. Once the tissue has been completely disrupted the racked tubes should be briefly subjected to centrifugation (2000g x 5 seconds) to remove any material from the head of the tubes. The caps can be removed and discarded and the racks placed back on the instrument to receive an additional aliquot of reagent. At the end of the automated lysis protocol, the racked tubes should be incubated in an oven (see off-line processing, p.9), briefly cooled and centrifuged before further processing on the instrument (see section on main automated process, p.10).

Disruption (Milling)

Mechanical disruption of tissue samples using a mixer mill (otherwise known as a bead beater or grinder) is essential for efficient DNA preparation. Each of the three lysis protocols must be used in conjunction with a mixer mill. On-screen instructions are provided during each protocol to advise when samples should be subjected to milling.

Prior to milling, the racked samples (without caps) must be placed on the instrument to receive lysis solution in the course of the automated lysis protocol. Tubes must then be sealed with push fit strip caps (provided) and placed in the mixer mill. Complete disruption of the plant tissue must be achieved (tissue structure should not be visible after milling) prior to further processing. Typically this can be achieved by using 2 x 1.5 minutes of milling, swapping the orientation of the block within the mill for the second period to promote reproducibility across the rack (consult manufacturer for advice or Tepnel Technical Services if required). A five second period of centrifugation at 2000 x g is required after milling to remove material from the caps before further processing (See off-line processing, p.9).

Notes

- The sample input tubes should never be immersed in liquid nitrogen to promote disruption during milling. It is not required and will result in split sample tubes.
- Sample tubes that will not be used within a run (i.e. they do not contain a sample) should be removed before placing the rack in a mixer mill (tubes may split if used in a mixer mill without reagents present).
- For tissues such as lavender leaves and pine needles it is essential that the elongated leaves are cut in < 3mm lengths in order that the ball bearings can efficiently impact on the tissue. Without this reduction the ball bearings will travel alongside the vertical samples resulting in a minimal level of disruption.
- Complete disruption is essential for high yields. Overloading can result in poor yield and quality through inefficient disruption.
- OVERLOADING CAN ALSO RESULT IN THE FILTER TIPS FAILING TO INSERT TO THE CORRECT DEPTH WITHIN THE SAMPLE TUBE THUS CAUSING THE UPPER X AXIS TO COLLIDE WITH THE PROTRUDING FILTERS
- For particularly coarse tissues such as pine needles, less than 50mg of material should be loaded as the disrupted material may force the filters up from the tubes post filtration leading to a collision with the upper X axis.

Now look at the Set-Up instrument screen and remove any Columns or Filters highlighted in black. Press OK on the screen once done.

3. TROUGH

Place the Dual Trough in **position 3**. The holder is designed to ensure the trough is located in the correct orientation. The larger Magnetic Particles Trough is positioned to the right and the RNase Trough is positioned to the left. Consult the Set-Up screen to obtain the correct fill volumes for the Dual Trough.

Magnetic Particles: Either pour or pipette the appropriate volume of Magnetic Particle/Isopropanol Suspension into the larger Magnetic Particles Trough (right hand side of the Dual Trough - the steps in the moulding of this trough indicate the following approximate fill volumes: 7.5mL, 15mL, 20mL and 25mL). For a run of 96 samples completely fill the trough.

RNase A: Pour or pipette an appropriate amount of RNase A/Isopropanol suspension (as shown on the instrument screen) to the smaller RNase Trough, (left hand side of the Dual Trough). For a run of 96 samples, completely fill the trough.

Note: The required volumes of RNase A and magnetic particle suspension can be found on the set up screen (see Instrument set up and operation: Main process, p.11)

Lower X Carriage Assembly:

4. WASH DEEP WELL PLATE

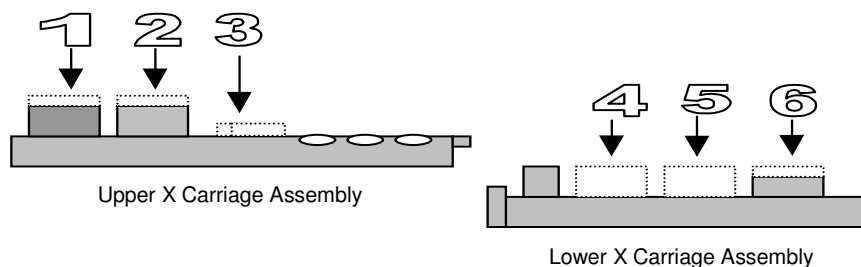
Place a clean, empty Deep Well Plate in **position 4. Well A1 to be placed in the rear, right-hand corner of position 4.**

5. SAMPLE RACK

Place your samples in racked tubes containing your lysed samples in **position 5**. The first sample should be placed in the rear right-hand corner of the input position (clearly labelled A1) and loaded in a back to front, right to left configuration. Ensure the rack is flat and secure. Gently push the bottom of the rack back up into place if it has become displaced during centrifugation in order to allow it to fit correctly into position.

LOADING CONFIGURATION:

Diagrammatic representation of the upper and lower X axes



Upper X Carriage Assembly:

1. COLUMNS

Remove the plastic skirt from around the Columns and insert the Columns in their carrier into the Column Block in **position 1**. Once the Columns are in position, push the carrier securely down onto the surface of the Block.

(Note: once empty the carrier must be removed before the next set of Columns is inserted.)

2. FILTER TIPS

Remove the plastic skirt from around the Filters and insert the Filter Tips in their carrier into the Filter Tip Block in **position 2**. Once the Filter Tips are in position, push the carrier securely down onto the surface of the Block ensuring that the carrier is pushed under the retaining clips.

(Note: Once empty the carrier must be removed before the next set of Filter Tips is inserted.)

NOTES:

- It is important that the Column and Filter Tip Carriers are securely located to prevent snagging when Columns/Filter Tips are picked up by the instrument.
- To avoid wastage of Columns and Filter Tips, ensure that the number and position of Columns and Filter Tips correspond with the number and position of input samples.

OFF LINE PROCESSING

After lysis and disruption using a mixer mill, samples require off-line incubation at an elevated temperature.

- An incubation at 65°C for 1 hour will maximise yield and quality. A minimum of 10 minutes is required.
- Alternatively, an overnight incubation at 50°C can be used to support a different work flow if required.

Note: An inverted rack lid is sufficient to act as a seal for periods of incubation up to an hour. Caps will be required to seal tubes to support the overnight lysis of samples using the standard or high MW lysis protocols.

After incubation, a 10 minute cooling period at –20°C is required. The samples should then be subjected to centrifugation for 1 minute at 2000 x g. The samples can then be processed immediately using the main protocols (see next section on main processing) or stored at 4°C for use at a later time during the same day.

NOTE: AFTER CENTRIFUGATION, THE BASE OF THE TUBE RACKS MAY HAVE DROPPED, REQUIRING THEM TO BE GENTLY PUSHED BACK UP INTO PLACE IN ORDER TO FIT CORRECTLY ONTO THE INSTRUMENT.

MAIN AUTOMATED PROCESS

After disruption and lysis are complete, the samples can be processed using the plant protocol (duration: 1.75 hours for 96 samples). An extended protocol is available which uses an extended washing regime (duration 2.5 hours) and may be used for “difficult samples” which may for example contain high levels of oils, polysaccharides, polyphenolics etc..

Both protocols provide walk away automation using a magnetic bead based process. In brief, the process consists of filtration, DNA binding, washing and elution. The DNA is eluted into 10mM Tris (EDTA is not present)

Storage

Typically eluted DNA may be stored at 4°C for a week. It is recommended that freezing at –20°C is used for longer time periods and –80°C for long term storage.

INSTRUMENT SET-UP AND OPERATION: LYSIS

Load the reagent bottles: The bottle positions on the instrument are labelled A-D corresponding with Reagents A-D. To load the reagents onto the instrument, unscrew the lids from the reagent bottles and replace them with the bottle connectors from the corresponding positions on the instrument (A–D). Depress the metal tab on each bottle connector to latch them open and then plug them onto the instrument using a push fit action. If the bottles will not connect properly, this may be because the tabs are not properly latched open. Ensure that the bottle connectors are properly engaged by gently tugging the bottles to ensure they will not disengage.

IMPORTANT: The bottle caps containing the connectors are not disposable. Do not throw them away.

Select run conditions: Switch on the instrument. The touch screen will display a splash screen, touch the screen and the Main Menu screen will appear. Click on the Select Protocol button and then select the required plant lysis protocol by navigating through the options.

- Select the option for use with kit No. 33000 (4 X 96) - The high usage protocols are for use with the 20 x 96 sample plant kit (No. 33100)
- To process one block select the single block lysis protocols
- To process two blocks in parallel, select the dual block lysis option
- Select from the standard, high MW, and Low MW protocols as required.

Place the sample rack into position 5 (indicated by the diagram on page 12) on the lower X axis. Ensure the rack is flat and secure. Gently push the bottom of the rack back up into place if it has become displaced during centrifugation in order to allow it to fit correctly into position.

Follow the instructions on the screen and input the number of samples to be processed (up to 96). The instrument will provide prompts for each step in this process.

A Set-Up screen will then appear and is followed by prompts to check the liquid and solid waste. Once complete, launch the protocol and follow the on-screen instructions which will advise when to add or remove racks from the instrument and when milling should occur. Once lysis and disruption is complete, the samples should be subjected to off-line processing (see off-line processing, p.9)

INSTRUMENT SET-UP AND OPERATION: MAIN PROCESS

Reagent bottles: Reagent bottles A - D should be present in positions A - D as described on page 10.

Select run conditions: Switch on the instrument. The touch screen will display a splash screen, touch the screen and the Main Menu screen will appear. Click on the Select Protocol button and then select the required plant lysis protocol by navigating through the options.

- Select the option for use with kit No. 33000 (4 X 96) - The high usage protocols are for use with the 20 x 96 sample plant kit (No. 33100)
- Navigate through the “Plant purification protocols (33000)” screen and select either the plant protocol or the extended plant protocol (see main automated process, p.9 for further details).
- The Nucleplex instrument can process one set of racked tubes at a time. Other racks that have been subjected to disruption, incubation and centrifugation should be stored at 4 °C until processing on the same day.

Once the desired parameters have been entered and confirmed, a screen showing the location of the Columns and Filter Tips will appear if less than 96 samples are to be processed. Using the arrow buttons, select the position of the first row of Columns and Filter Tips to be used (Black represents empty positions, Red represents the location of Columns and Filters that will be used during the protocol and Orange represents positions that may be optionally occupied or empty).

A Set-Up screen will then appear which provides information on the reagents and plasticware required to complete the protocol. Ensure that the required plasticware and reagent volumes are loaded in the indicated positions prior to starting the instrument (see Loading Configuration, p.12).

Check that sufficient capacity is available in the waste bin and liquid waste bottle to receive solid and liquid waste during the run.