

WARRANTY

Nucleoplex Plasmid DNA Extraction Kits come with a 'no quibble' warranty. We trust that this Nucleoplex 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
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)
33210	Nucleoplex BAC High Usage kit for up to 1920 extractions (plasticware 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.

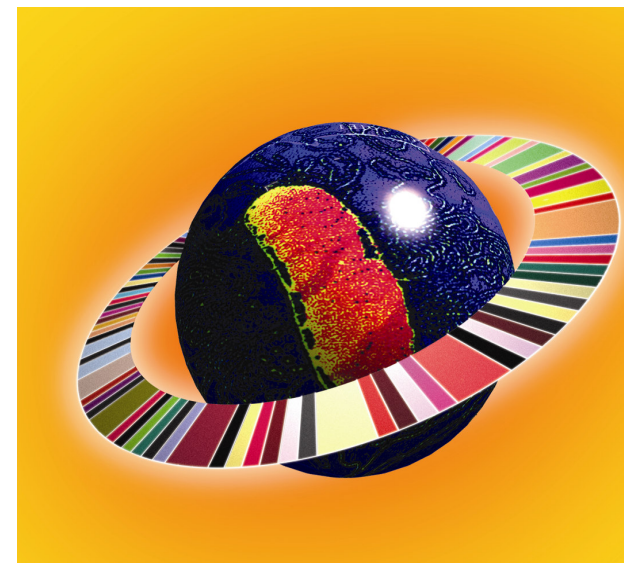
BigDye™ is a trademark of Applied BioSystems.

NUCLEOPLEX™ PLASMID DNA KIT

Product No. 33100

(2 x 96 preps)

NB: Please refer to kit contents section for storage instructions



UK Tel: +44 (0)161 946 2222
USA Tel: +1 (888) 329 0255
Email: support@tepnel.com

UK Fax: +44 (0)161 946 2224
USA Fax: +1 (203) 328 9599
www.tepnel.com

Heron House, Oaks Business Park, Crewe Road, Wythenshawe,
Manchester, M23 9HZ. UK



UK Tel: +44 (0)161 946 2222
USA Tel: +1 (888) 329 0255
Email: support@tepnel.com

UK Fax: +44 (0)161 946 2224
USA Fax: +1 (203) 328 9599
www.tepnel.com

Heron House, Oaks Business Park, Crewe Road, Wythenshawe,
Manchester, M23 9HZ. UK

INTENDED USE:

This kit is for research use only and is to be used solely for the intended purpose of extracting plasmid DNA from bacterial cell pellets arising from the culture of Gram negative *E.coli* strains containing low to high copy number plasmids.

KIT CONTENTS:

Kit Component	Number of Bottles	Storage	Product Number
Lysis Buffer (A) ~95mL	1 x 125mL	15-25°C	33100/03
Wash Buffer (B) ~50mL (each bottle)	2 x 250mL	15-25°C	33100/05
Neutralisation Buffer (C) ~86mL	1 x 125mL	15-25°C	33100/04
Resuspension/Elution Buffer (D) ~265mL	1 x 250mL	15-25°C	33100/06
Binding Solution ~55mL	1 x 60mL	15-25°C	33100/09
Magnetic Particles ~5mL	1 x 8mL	15-25°C	33100/08
RNase A Solution ~0.26mL	1 x 0.5mL	15-25°C	33100/07
Deep Well Plate	4	N/A	P0080
Microtitre Elution Plate	2	N/A	P0093
Plate Sealing Membranes	2	N/A	30011
Columns (Racked)	2 x 96	N/A	R0013
Filter Tips (Racked)	2 x 96	N/A	R0012
Plate Gas Permeable Membranes	2	N/A	30012

ITEMS REQUIRED BUT NOT SUPPLIED:

- 2 x 200mL analytical grade ethanol (greater than 96%) is required to make up the Plasmid Wash Buffer.

See page 9 for supplier information.

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 2 x 96 samples. If multiple extractions of less than 48 samples are carried out, additional reagents may be required. Please contact your local supplier for more information.
- Lysis Buffer at temperatures below 15°C may form a precipitate. Re-dissolve before use by heating to 30°C for 5 minutes.

PRECAUTIONS:

- Lysis Buffer contains sodium hydroxide (NaOH) and sodium dodecyl sulphate (SDS) 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 NaOH and SDS 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.
- Add 200mL of (96%) ethanol to reagent Bottle B (Wash Buffer). The fill level is indicated by the marked label.
- Ensure the Magnetic Particle Suspension is completely mixed by shaking or vortexing thoroughly. Pour all the contents of the bottle containing the Magnetic Particles into the Binding Solution (the neck of the smaller bottle will fit into the larger bottle to aid this process). 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 vortexed or shaken before use. The combined reagent is assigned a shelf life of 6 months at 15-25°C.

- **Once opened, and made up, Reagents A-D have a shelf life of 3 months at 15°C-25°C.**

CELL CULTURE PREPARATION:

- Transfer 1.3mL of culture medium* containing selective antibiotic into the required number of wells of an empty 96 Deep-Well Plate (Sample Deep Well Plate). Wells should be filled from well A1 and by row. *Tepnel recommends that enriched media such as TB are used to maximise plasmid DNA yields.
- Inoculate each well with a bacterial colony picked from a fresh stock.
- Cover the plate with a gas permeable membrane.
- Grow with shaking for 20-22 hours at 300rpm in 37°C incubator (growth characteristics of *E.coli* host/culture medium combinations will vary).
- Alternatively, the culture can be grown in another container using your standard conditions and 1.5mL aliquots transferred to each well of the Deep Well Plate.
- Check the cell culture OD₆₀₀ of 1/10 dilutions to determine the Biomass (A₆₀₀ x dilution factor x mL culture). A Biomass of 3-10 is recommended for best results.

SAMPLE PREPARATION (1.0mL-1.5mL cell cultures):

Cell cultures should be pelleted by centrifugation for 2 x 3 minutes at 1500 x g prior to loading on the instrument. The Deep Well Plates should be turned through 180° after first 3 minute spin step.

Input samples are loaded onto the instrument as bacterial pellets in a 2mL Sample Deep Well Plate.

For further information on host, vector and culture conditions please refer to the Nucleplex plasmid purification applications note, which may be obtained from your supplier or www.tepnel.com.

INSTRUMENT SET-UP AND OPERATION:

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

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. DIS-984-010N

www.nalgenunc.com

Gas Permeable Membranes Web Scientific

Cat. No. WTS-7014

www.webscientific.co.uk

Plate Sealing Membranes Invitrogen

Cat. No. DIS-984-505J

www.invitrogen.com

Remove samples: A Protocol Complete screen is displayed once the protocol has ended and the Elution Plate may be removed. Following the standard Plasmid protocol the Elution Plate should be removed for storage in appropriate conditions (4°C for short-term storage, -20°C for long-term storage). Use a membrane to seal the plate prior to storage. The Nucleplex Plasmid Automated Purification protocol produces DNA that is ready for use in most applications, including capillary electrophoresis sequencing and PCR[†]. Following the rapid protocol the Elution Plate will require further incubation at room temperature (15-25°C) for 2-4 hours to ensure that any residual ethanol has evaporated, prior to use in downstream applications.

Waste removal: At the end of the run remove all used plasticware from the instrument. 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 192 samples in up to 4 runs. The instrument allows any number of plasmid 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.

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.

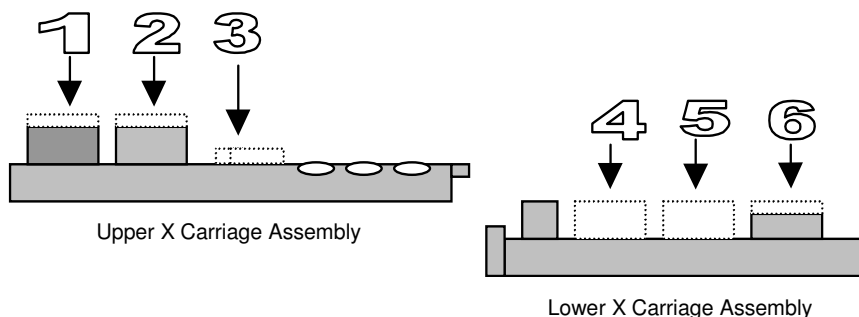
Select run conditions: Switch on the instrument. The touch screen will display a splash screen, touch the screen and the Main Menu screen will appear. Click on the Select Protocol button and then select the required protocol. Input the number of samples to be processed and the desired elution volume on the relevant screens. The instrument will provide prompts for each step in this process.

Once the desired parameters have been entered and confirmed, a screen showing the location of the Columns and Filter Tips will appear. 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 below).

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

LOADING CONFIGURATION:



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.
- 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 right and the RNase Trough is positioned 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 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).

RNase A: Using a suitable pipette (e.g. 100µL or 200µL volume), add the recommended volume of RNase A (as shown on the instrument screen) to the smaller RNase Trough (left hand side of the Dual Trough) spreading it evenly along the bottom of the V section. For small numbers of samples (less than 12), the RNase A should be spread only within the region of the trough corresponding to the sample position.

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 DEEP WELL PLATE

Place your samples in a Deep Well Plate 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.

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 plasmid DNA is eluted into the Microtitre Elution Plate at the end of the run. The well position of the purified plasmid 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 and click on the Run button. Throughout the run, the touch screen displays the status of the protocol in large text.