

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 Nucleon range:

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
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

Please contact your local supplier for further information.

NUCLEON[®] Mouse Tail DNA Extraction Kits 44200

For Research Use Only

Instructions for Use

NUCLEON[®] is a registered trademark of Gen-Probe Life Sciences Ltd.

NUCLEON kits are manufactured by Gen-Probe Life Sciences Ltd. within quality systems certified to ISO9001 and ISO13485

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Nucleon kits have been used by scientists for many years and have consistently provided high yields of highly pure, highly intact DNA from a range of sample types. In response to concerns over the use and disposal of chloroform, a chloroform-free version of the chemistry has been developed.

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 ≥ 1.80 .

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

Safety: This kit generates no chlorinated waste by-products

TYPICAL YIELDS

This kit operates most effectively over the sample weight range 2.5mg-20mg. Yields are typically 2.0 μ g-5.0 μ g DNA per mg of starting sample. The DNA has a high molecular weight and shows little shearing, rendering 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 mouse tails.

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

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 fall in the range 1.7-1.9. 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. In addition, excessive protein might not be efficiently precipitated out of the solution. Therefore the sample should be split and the recovered DNA pooled at the end of the process.

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 longer to achieve the same effect. For example, 1000g for 10 minutes gives much the same total effect as 2000g for 5 minutes.

2. *Nucleon Resin carry over into the DNA pellet*

It is possible for some Nucleon resin to be carried over into the DNA pellet, which then appears brown/red in colour. This will not happen if the protocol is carefully followed and if the correct centrifugation speeds are used (refer to the formulae below). In the event of carryover, the Nucleon resin has been demonstrated not to interfere with downstream processes such as PCR¹, however, if removal is required, a brief centrifugation at 3000g should pellet any resin present.

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

This will occur 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 resuspension solution.

KIT CONTENTS

Component	Number of Bottles	Storage Conditions on arrival
Proteinase K (10mg)	1	-20°C
Reagent M1 (10mL)	1	15-25°C
Reagent M2 (4mL)	1	2-8°C
Nucleon Resin (5.5mL)	1	15-25°C
Protocol Booklet	N/A	-

Additional materials required but not supplied:

Ethanol, propan-2-ol, water, TE Buffer, RNase (optional)

SAFETY

The Nucleon extraction kit component Reagent M2 contains a chemical reagent that may be hazardous. This chemical is irritating to eyes, respiratory system and skin. It 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. 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. The Proteinase K is supplied as a powder (10mg) which should be dissolved in 1mL of sterile water and dispensed into 5 x 200µL aliquots. These should be stored at -20°C. Once thawed, individual aliquots should be stored at 2°C - 8°C for no longer than 1 week before being discarded.

STORAGE/STABILITY

Nucleon kits are stable at the storage temperatures indicated above for 12 months (3 months once opened) if handled under aseptic conditions. If Reagent M1 is stored below 15-25°C white crystals may form. These will dissolve on warming.

PROTOCOL (Sample weight range 2.5mg-20mg)

Tissue Preparation and lysis

1. Add approximately 1cm (2.5-20mg) of mouse tail to a 1.5mL microtube.
2. Add 175µL of Reagent M1 to the tube.
3. Add 20µL of the 10mg/mL Proteinase K solution, seal the tubes and mix by inversion.
4. Ensure that the tail is covered by the solution.
5. Incubate the tubes at 55°C for at least 8 hours or overnight.

Deproteinisation

1. Mix the tubes by inversion at least 10 times on removal from the incubator.
2. Add 65µL of Reagent M2 and 100µL of Nucleon Resin suspension to the tubes ensuring that the resin is fully re-suspended before use.
3. Mix the tubes by inversion at least 10 times.
4. Centrifuge the tubes at 2000g for 10 minutes.
5. On removal of the tubes from the centrifuge, if there is residual tissue debris on top of the resin layer mix by inversion 20 times and re-spin the tubes at ~10,000g for 1 minute (usually top speed on a standard centrifuge).

DNA precipitation

1. Without disturbing the Nucleon Resin/debris layer, transfer the aqueous phase to a clean 1.5mL microtube.
2. Add 1 volume of 100% propan-2-ol. Invert the tube several times until the DNA precipitate is visible.
3. Centrifuge the samples at 2000g for 5 minutes to pellet the DNA.
4. Carefully discard the supernatant without disturbing the DNA pellet.

DNA washing

1. Add 200µL of 70% ethanol and mix the tube contents to wash the DNA pellet. Make sure the pellet is dislodged from the bottom of the tube during this process. Re-centrifuge the samples at 2000g for 2 minutes, and discard the supernatant.
2. Air-dry the pellet at room temperature for 10 minutes. Do not exceed this time, as this may render the DNA more difficult to dissolve.
3. Resuspend the DNA in TE buffer or water (about 300µL), as required.
4. Allow at least 3 hours at room temperature for the DNA to re-hydrate.

HELPFUL HINTS

Note 1:

The Nucleon extracted DNA may contain small amounts of RNA, which should not interfere with DNA amplification or restriction digest. If RNA-free DNA is required, an RNase A digestion step should be included. RNase should be made up in water and boiled for 10 minutes to inactivate any DNase. Add 7.5µL of a 50µg/mL RNase A solution and incubate at 37°C for 30 minutes. It is best to perform RNase treatment after the addition of Reagent M1.

Note 2:

The resin layer should not be disturbed in order to minimise contamination. However, the resin itself, if carried over, will not interfere with subsequent processing of the DNA.

TROUBLESHOOTING

1. Low Yield of DNA

Possible causes at the Cell Lysis Stage:

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

The tail sample needs to be of a reasonable size (2.5-20mg) and quality (the sample should contain tissue as opposed to just hair or bone). Check the sample size prior to cell lysis step to ensure that the sample falls within the recommended range. If a low yield is expected due to extremely small sample size, recovery may be enhanced by adding a carrier DNA (e.g. denatured herring sperm DNA) or glycogen (1µL of 20mg/mL glycogen per 600µL propan-2-ol).

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

This may result in incomplete lysis due to overloading of the chemistry. Split the sample as appropriate and proceed with the protocol ensuring that sufficient proportional volumes of Reagents M1 and M2 are used to prevent overloading of the chemistry.

3. *Incomplete cell lysis*

Incomplete cell lysis can occur due to overloading (see above) or due to the detergent in Reagent M1 coming out of solution. The detergent can be re-dissolved by heating Reagent M1 to 37°C for 10 minutes prior to use.