

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:

35100	Nucleopure Mouse Tail kit for up to 960 extractions
35200	Nucleopure QD Mouse Tail kit for up to 960 extractions

Kits available in the Nucleoplex™ range:

33100	Nucleoplex Plasmid kit for up to 192 extractions
33200	Nucleoplex BAC kit for up to 192 extractions
33201	Nucleoplex BAC kit for up to 192 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.

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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

HARD TISSUE

Product number: SL 8509

For 50 preps of up to 25mg hard tissue or paraffin-embedded sections

NB: Please refer to kit contents section for storage instructions



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Nucleon kits, employing the patented Nucleon Resin, 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.

Nucleon HT protocols have been developed for those tissues which do not homogenise easily in lysis buffer and require a proteinase K digest. Such tissues are exemplified by paraffin embedded tissue segments, mouse tails and xiphisternum.

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 phenol waste.

TYPICAL YIELDS

This kit operates most effectively over the sample weight range 10mg-60mg.

Sample	Yield DNA	Purity $A_{260/280}$ (average)
Mouse tail	>40 μ g/cm	≥ 1.8
Skin	1.4 μ g/mg	≥ 1.8
Xiphisternum	1.6-1.9 μ g/mg	≥ 1.8

1 cm of mouse tail equates to approximately 60mg of tissue.

INTENDED USE

This kit is intended for research use only for the extraction of DNA from hard tissue e.g. mouse tails, skin and xiphisternum.

KIT CONTENTS

	SL8509	Number of Bottles	Storage conditions on arrival
Reagent B	18mL	1	15-25°C
Sodium Perchlorate	6mL	1	15-25°C
Nucleon Resin	8mL	1	15-25°C
Proteinase K	10mg	1	-20°C
Protocol Booklet	-	N/A	-

Additional materials required but not supplied:

Chloroform, ethanol, glycogen (optional), water, TE Buffer, RNase (optional)

SAFETY

The Nucleon extraction kit component sodium perchlorate is 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. 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. 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 2-8°C for no longer than 1 week before being discarded.

STORAGE/STABILITY

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

PROTOCOLS

PROTOCOL 1 For 50 preps of up to 25mg of hard tissue

Tissue preparation and lysis

1. Finely chop and grind 25mg tissue on dry ice or in liquid nitrogen to a fine powder and transfer to a 1.5mL microtube. Add 350µL of Reagent B.
2. Optional RNase A treatment: add RNase to a final concentration of 400ng/mL and incubate at 37°C for 30 minutes.
3. Add 18µL of the Proteinase K solution and incubate at 50-55°C for at least three hours (or leave overnight).
4. Centrifuge at 2000g for 5 minutes.
5. Remove the supernatant and transfer to a fresh 1.5mL microtube for the next stage.

Deproteinisation

1. Add 100µL sodium perchlorate and mix by inverting at least 7 times. It is strongly recommended that this is done by hand.
2. Add 600µL chloroform and mix by inverting at least 7 times. It is strongly recommended that this is done by hand.
3. Add 150µL Nucleon Resin suspension, rotary mix for 5 minutes and centrifuge at 350g for 1 minute.

DNA precipitation

1. Without disturbing the Nucleon Resin layer (brown in colour), transfer the upper phase to another 1.5mL microtube.
2. If any resin has been carried over, centrifuge briefly and then transfer to another 1.5mL microtube.
3. Add 2 volumes of cold absolute ethanol and invert several times until the DNA is precipitated.

DNA washing

1. Centrifuge at top speed (minimum of 4000g) for 5 minutes to pellet the DNA then discard the supernatant.
2. Add 1mL cold 70% ethanol and invert several times. Re-centrifuge and discard the supernatant. This step can be repeated if necessary.
3. Air-dry the pellet for 10 minutes, ensuring that all the ethanol is removed and resuspend in TE or sterile water. The DNA should re-dissolve within 2 hours if placed on a rotary mixer.

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

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 extraction.

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.

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 PCR[†], 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 try heating samples to 65°C for 2-3 hours after addition of the resuspension solution.

PROTOCOL 2 For 50 preps of up to 25mg from paraffin-embedded sections

Preparation of paraffin sections

1. Take one 20-30µm section of tissue in a microtube.
2. Incubate in xylene at 37°C for 20 minutes. Centrifuge at 1300g for 5 minutes and remove the xylene.
3. Incubate in xylene at room temperature for 2 minutes. Centrifuge at maximum speed in a microfuge and remove the xylene.
4. Rehydrate with consecutive washes of 100% ethanol, 75% ethanol, 50% ethanol, 25% ethanol and water, spinning at maximum speed in a microfuge between each wash. At the 25% ethanol and water stages, care should be taken as the material may become loose and difficult to pellet.
5. Pour off the water and add 350µL Reagent B to the microtube. Add 18µL Proteinase K solution and incubate at 55°C for 3 hours or overnight if preferred.

Deproteinisation

1. Add 100µL sodium perchlorate and mix by inverting at least 7 times. It is strongly recommended that this is done by hand.
2. Add 600µL chloroform and mix by inverting at least 7 times to emulsify the phases. It is strongly recommended that this is done by hand.
3. Add 150µL Nucleon Resin suspension and, without re-mixing the phases, centrifuge at 350g for 1 minute.

DNA precipitation

1. Without disturbing the Nucleon Resin layer (brown in colour), transfer the upper phase to another 1.5mL microtube.
2. If any resin has been carried over, centrifuge briefly and then transfer to another 1.5mL microtube.
3. Add 2 volumes of cold absolute ethanol and 1µL of 20mg/mL glycogen solution if required as a carrier. Leave at -20°C for 1-2 hours to precipitate the DNA.

DNA washing

1. Centrifuge at top speed for 15 minutes to pellet the DNA then discard the supernatant.
2. Add 1mL cold 70% ethanol and invert several times. Recentrifuge and discard the supernatant. This step can be repeated if necessary.
3. Air-dry the pellet for 10 minutes and resuspend in TE or sterile water (e.g. 100µL). The DNA should re-dissolve within 2 hours if placed on a rotary mixer.

HELPFUL HINTS

Note 1:

For mouse tails, it may be necessary to grind the tissue with a mortar and pestle after finely chopping.

Note 2:

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, the RNase digestion step should be included. RNase A (not supplied) 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. Should RNA still be found to be contaminating the DNA preparation, increase the RNase concentration as appropriate. It is best to perform the RNase treatment after addition of Reagent B.

Note 3:

A centrifugal force of 350g corresponds to 2000rpm in an Eppendorf 5415 microfuge. Check the manual accompanying your machine or refer to page 11, Calculation of Centrifugal Force. Speeds higher than this may cause the resin to spin to the bottom of the tube.

Note 4:

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

Note 5:

Precipitated DNA may be hooked out using a heat-sealed Pasteur pipette. This DNA does not require a 70% ethanol wash and should be placed directly into TE or sterile water.

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 size needs to be of a reasonable size (10-60mg) 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 sample).

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

If this is the case, incomplete lysis may occur due to overloading of the chemistry. Split the sample as appropriate and proceed with the protocol ensuring that sufficient proportional volumes of Reagents B and C are used to prevent overloading of the system.

3. *Incomplete cell lysis*

Incomplete cell lysis can be due to the detergent in Reagent B coming out of solution. The detergent can be re-dissolved by heating to 37°C for 10 minutes prior to use.

Possible causes post extraction:

1. *Inaccurate absorbance measurement*

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