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® BACC DNA Extraction Kits SL8502 and SL8512

For Research Use Only

Instructions for Use

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

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Nucleon kits, employing the patented Nucleon chemistry, have been used by scientists for many years and have consistently provided high yields of highly pure, largely intact DNA from a range of sample types.

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 by-products.

TYPICAL YIELDS

This kit operates most effectively over the sample range 250mL-10mL.

Sample	Yield DNA	Purity $A_{260/280}$ (average)
Whole Blood	370–440g/10mL	1.8-2.1
HeLa Cells	12µg /10^6 cells	1.8

INTENDED USE

This kit is intended for research use only for the extraction of DNA from whole blood and cultured cells. Buccal cells and Buffy coat preparations can also be processed using this kit.

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.

$$g = 1.12r \left(\frac{r.p.m}{1000} \right)^2$$

$$r.p.m = 1000 \sqrt{\frac{g}{(1.12r)}}$$

r = maximum radius of rotor in mm

Additional Reagent A preparation for Nucleon BACC2 Kits

10mM Tris-HC1, 320 mM sucrose, 5mM $MgCl_2$, 1% (v/v) Triton X-100, pH 8.0

Combine reagents in 80% of the volume required. Mix to dissolve. Adjust pH to 8 using 40% (w/v) NaOH. Make up to volume and mix well. Autoclave suitable aliquots at 121°C 15 psi for 15 minutes.

3. 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 within the range of 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 the excess 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 as 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 is unlikely to 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 occurs when 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 re-suspension solution.

KIT CONTENTS

	BACC2 mL (SL8502)	BACC3 mL (SL8512)	Number of Bottles	Storage Conditions on arri-val
Reagent A 1x	420	-	1	15-25°C
Reagent A 4x	-	510	1	15-25°C
Reagent B	110	110	1	15-25°C
Sodium Perchlorate	26	26	1	15-25°C
Nucleon Resin	16	16	1	15-25°C
Protocol Booklet	-	-	N/A	-

Additional materials required but not supplied:

Ethanol, chloroform, water, TE Buffer, RNase (optional).

SAFETY

All Nucleon extraction kit components contain chemical reagents that may be hazardous. These chemicals are irritating to eyes, the respiratory system and skin. They 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. Reagent A in BACC 3 is supplied as a 4 x concentrate. Prior to use, it must be diluted in the ratio 3:1 with de-ionised water and then autoclaved.

STORAGE/STABILITY

Nucleon kits are stable at the storage temperatures indicated above for 36 months (3 months once opened) if handled under aseptic conditions. Reagent A once opened must be stored at 2-8°C.

BLOOD COLLECTION

Blood collected in potassium EDTA tubes is suitable for genomic DNA extraction using this kit. Heparinised or citrated blood is also suitable.

PROTOCOLS

1 For small blood volumes (up to 1mL) and $1x10^6$ to $3x10^6$ cultured cells.

Cell preparation from whole blood

1. Collect blood in sodium EDTA tubes (heparinised or citrated blood is also suitable).
2. To 250µL–1.0mL blood, add 4 times the volume of Reagent A.
3. Rotary mix/shake for 4 minutes at room temperature then centrifuge at 1300g for 5 minutes. Discard the supernatant without disturbing the pellet.

For Buffy coat or Buccal cell preparation **see Helpful Hint 6.**

OR

Cell preparation from 1 x 10^6 to 3 x 10^6 cultured cells

1. Collect the cultured cells by centrifugation at 600g for 5 minutes at 4°C.
2. Discard the supernatant without disturbing the pellet.
3. Resuspend cells in 1.0mL of Reagent A and leave on ice for 5 minutes.
4. Centrifuge at 1300g for 5 minutes at 4°C and discard the supernatant.

Cell lysis

1. To the pellet, add 350µL of Reagent B (ensuring that the detergent is fully dissolved) and vortex briefly to resuspend the pellet. Incubating the samples at 37°C for 10 minutes can also help to resuspend the pellets.
2. Transfer the suspension to a 1.5mL microtube.
3. Optional RNase treatment: add 2.5µL of a 50µg/mL RNase A solution and incubate at 37°C for 30 minutes.

Deproteinisation

1. Add 100µL of sodium perchlorate solution and mix by inverting at least 7 times. It is strongly recommended that this is done by hand.
2. Add 600µL of chloroform. Mix by inverting at least 7 times to emulsify the phases. It is strongly recommended that this is done by hand.
3. Add 150µL of Nucleon Resin and without re-mixing the phases, centrifuge at 350g for 1 minute. 350g corresponds to approximately 2000rpm in a standard microfuge.

2. Low yield of DNA

Possible causes at the white cell lysis stage:

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

Check the cell count 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) e.g. denatured herring sperm DNA or glycogen (1µL of 20 mg/mL glycogen per 600µL propan-2-ol).

2. Too many white cells present in the starting sample

Check the cell count. If there are too many cells, split the sample as appropriate and proceed with the protocol ensuring that sufficient proportional volumes of Reagents A, B, and C are used to prevent overloading of the chemistry.

3. *White cell nuclei pellet formation and/or pellet loss*

Pellets are more likely to dislodge from round-bottomed tubes than with centrifuge/conical-bottomed tubes. Therefore, use conical-bottomed tubes or take extra care when decanting the supernatant.

Normally with small blood samples (<5mL) it is difficult to see the white cell nuclei pellet at the bottom of the tube. Therefore, take care when removing Reagent A. If in doubt, use a Pasteur pipette and carefully drain the remaining Reagent A on to a tissue.

4. Incomplete cell lysis

Incomplete cell lysis can usually be attributed to two causes. The first is when the detergent in Reagent B comes out of solution. This can be resolved by heating Reagent B to 37°C for 10 minutes. The second cause of incomplete cell lysis occurs when the pelleted material clumps. Ensuring the pellet is fully re-suspended to homogeneity in Reagent B by vigorous vortexing or extended incubation at 37°C or room temperature can reduce this problem.

Possible causes post extraction:

1. Inaccurate absorbance measurement

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

Microfuge. Check the manual accompanying your machine or refer to Calculation of Centrifugal Force below. Speeds higher than this may cause the resin to spin to the bottom of the tube.

Note 5:
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 6:
Buccal cells/Buffy coat preparations: Cells harvested from buccal swabs/ spatulas in appropriate media (e.g. Isotonic saline, sucrose etc) and buffy coat preparations can be processed using the BACC protocols from the cell preparation stage onwards.

Note 7:
The extraction has been optimised using polypropylene centrifuge tubes. (15mL, screw-capped maximum internal diameter 12mm). All tubes used should have leak proof caps to maximise recovery.

Note 8:
Precipitated DNA may be hooked out after precipitation with propan-2-ol 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. Red colouration of samples

1. *Incomplete lysis of red blood cells*

This occurs either when there is an exceptionally high red cell count in the blood sample or, when white cell clumping around red cells prevents red cell lysis. These situations can be overcome by repeating the red cell lysis reaction with Reagent A.

2. Heme carry-over

This usually manifests itself as a red colouration of the white cell pellet. The likely cause of this is a low quality blood sample usually as a consequence of poor collection tube mixing or storage. In most cases this colouration will disappear upon continuation of the protocol to the point of DNA washing. There will be no effect upon the suitability of the extracted DNA for further enzymatic analysis by restriction, PCR etc. If this is not the case, repeat the Reagent A treatment as stated in the protocol.

DNA precipitation
1. Without disturbing the Nucleon Resin layer (brown in colour), transfer the upper phase (~ 450µL) to a new 1.5mL tube.
2. If any resin has been carried over, centrifuge briefly at a minimum of 1300g to pellet the resin and then transfer to another 1.5mL microtube.
3. Add approximately 2 volumes (900µL) of cold absolute ethanol and invert several times until the DNA is precipitated.

DNA Washing
1. Centrifuge at minimum 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 has been removed. Re-dissolve the DNA in an appropriate volume of water or TE buffer (e.g. 50-250µL). The DNA re-dissolves within 2 hours if placed on a rotary mixer.

2 Up to 10mL whole blood and $3x10^6$ - $1x10^7$ cultured cells

Cell preparation from whole blood

1. Collect blood in sodium EDTA tubes (heparinised or citrated blood is also suitable).
2. To a 50mL polypropylene centrifuge tube, add blood (3-10mL) and 4 times the volume of Reagent A.
3. Rotary mix/shake for 4 minutes at room temperature then centrifuge at 1300g for 4 minutes. Discard the supernatant without disturbing the pellet.

OR

Cell preparation from 3 x 10^6 to 1 x 10^7 cultured cells

1. Collect the cultured cells by centrifugation at 600g for 5 minutes at 4°C.
2. Discard the supernatant without disturbing the pellet.
3. Resuspend cells in 1.0mL of Reagent A and leave on ice for 5 minutes.
4. Centrifuge at 1300g for 5 minutes at 4°C and discard the supernatant.

Cell lysis

1. To the pellet, add 2.0mL Reagent B (ensuring that the detergent is fully dissolved) and vortex briefly to resuspend the pellet. Incubating the samples at 37°C for 10 minutes can also help to resuspend the pellets.
2. Transfer the cell suspension to a screw-capped polypropylene centrifuge tube (maximum internal diameter 12 mm).
3. Optional RNase A treatment: add 15µL of a 50µg/mL RNase A solution and incubate at 37°C for 30 minutes.

Deproteinisation

1. Add 500µL of sodium perchlorate and mix by inverting at least 7 times. It is strongly recommended that this is done by hand.
2. Add 2mL of chloroform. Mix by inverting at least 7 times to emulsify the phases. It is strongly recommended that this is done by hand.
3. Add 300µL of Nucleon Resin and without re-mixing the phases, centrifuge at 1300g for 3 minutes.

DNA precipitation

1. Without disturbing the Nucleon Resin layer (brown in colour), transfer the upper phase to a fresh tube of minimum volume 9mL.
2. If any resin has been carried over, centrifuge briefly at 1300g to pellet the resin and then transfer to a fresh tube.
3. Add two volumes of cold absolute ethanol and invert several times until the DNA is precipitated.

DNA Washing

1. Centrifuge at a minimum of 4000g for 5 minutes to pellet the DNA then discard the supernatant.
2. Add 2mL 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 all the ethanol has been removed and resuspend in TE or sterile water (e.g. 1.0–2.0ml). The DNA should re-dissolve in 2 hours if placed on a rotary mixer.

HELPFUL HINTS

Note 1:

In order to minimise damage to DNA in blood samples, stored at 4°C they should be extracted within 24 hours of collection. It has been reported that heparin can bind to DNA during extraction and if present in the final DNA solution, can interfere with downstream PCR† techniques (*Interference of heparin with the Polymerase Chain Reaction,* Biotechniques, (1990), Vol. 9, p166.) The effect of heparin can be counteracted in several ways and users of heparinised blood may find the following papers useful:

- *Optimisation of PCR to yield successful amplification from heparin-containing DNA.* Methods in Molecular and Cell Biology (1995), Vol. 5, pp122-124.
- *A rapid and simple method for reversing the inhibitory effect of heparin on PCR for HLA class 2 typing.* (1993) PCR methods and Applications, Chapter 2, pp356-358. Cold Spring Harbor ISSN 10549803/93.
- *Detection of mouse mast cell associated protease mRNA: Heparinase treatment greatly improves RT-PCR of tissues containing mast cell heparin.* (1995) American Journal of Pathology, Vol. 146, No. 2, pp335-343.

Note 2:

Reagent A contains high sucrose content and could be contaminated if aseptic technique is not used when dispensing.

Note 3:

The Nucleon extracted DNA may contain small amounts of RNA which should not interfere with DNA amplification or restriction. If RNA-free DNA is required, the RNase A digestion step should be included. A 50µg/mL RNase solution should be made with water and boiled for 10 minutes to inactivate any contaminating DNase. It is best to perform RNase treatment after addition of Reagent B.

Note 4:

A spin speed of 350g corresponds to 2000rpm in an Eppendorf 5415