# NUCLEON® Blood Non Chloroform DNA Extraction Kits
# 44100

## 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

Manufactured by Gen-Probe Life Sciences Ltd.
Heron House
Oaks Business Park
Crewe Road
Wythenshawe
Manchester
M23 9HZ
United Kingdom

For Sales, Customer Service and Technical Support:-

T: +49 (0) 6122 7076 451
F: +49 (0) 6122 7076 155
E: customerservice@gen-probe.eu
E: technicalsupport@gen-probe.eu

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 while maintaining performance. This kit has also been optimised to efficiently recover DNA, suitable for a variety of molecular biological applications, from fresh, frozen or poorly stored samples.

**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 1.80-1.90.

**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 volume range 0.5mL–10mL. Yields are typically 35µg–40µg DNA per mL of whole blood (dependent on the WBC count). Typically, high molecular weight DNA fragments are recovered showing minimal shearing, making it suitable for a range of downstream applications or for long term storage.

## INTENDED USE

This kit is intended for research use only for the extraction of DNA from whole human blood.



## 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.

## 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



## KIT CONTENTS

| Components | Number of Bottles | Storage Conditions on arrival |
|---|---|---|
| Reagent A (450mL) | 3 | 15-25°C |
| Reagent B (55mL) | 1 | 15-25°C |
| Reagent C (20mL) | 1 | 2-8°C |
| Nucleon Resin (16mL) | 1 | 15-25°C |
| Protocol Booklet | N/A | - |

**Additional materials required but not supplied:**
Ethanol, propan-2-ol, 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.
All reagents are supplied ready-to-use.

## STORAGE/STABILITY

Nucleon kits are stable at the storage temperatures indicated above for 12 months (3 months once opened) if handled under aseptic conditions. Once opened, Reagent A should 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.

## IMPORTANT

- For best results always use the protocol that is most appropriate for the sample volume you wish to process.
- Ensure that all reagents are in solution prior to use.

## PROTOCOLS

### PROTOCOL 1 (3.1mL-10mL)

### Cell Preparation from Whole Blood

1. For volumes <5mL add the blood sample to a 15mL polypropylene centrifuge tube. For volumes > 5mL add the blood sample to a 50mL polypropylene centrifuge tube.
2. Add 2 volumes of Reagent A.
3. Rotary mix/invert for 4 minutes at room temperature then centrifuge at 3500g for 5 minutes. Discard the supernatant without disturbing the pellet.
4. Add 5mL of Reagent A. Vortex/mix to resuspend the cell pellet. This should take about 1 minute.
5. Centrifuge at 3500g for 5 minutes. Discard the supernatant without disturbing the pellet.

### Cell Lysis

1. To the pellet add 1mL of Reagent B and vortex/mix to resuspend the cell pellet. This should take about 1 minute.
2. If you experience difficulty in resuspending the pellet, it may help to incubate the samples for 10 minutes at 37°C.

### Deproteinisation

1. Add 350µL of Reagent C and mix by inverting at least 7 times.
2. Add 300µL of Nucleon Resin drop-wise to the top of the sample and without further mixing, centrifuge at 3500g for 4 minutes.

### DNA Precipitation

1. Without disturbing the pellet, transfer the supernatant to a clean 15mL polypropylene centrifuge tube.
2. Add 1 volume of 100% propan-2-ol to the recovered supernatant and invert the tube several times until the DNA precipitate is visible.
3. Centrifuge at 4000g for 5 minutes to pellet the DNA then discard the supernatant.

### DNA Washing

1. Add 1mL 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 at 4000g for 2 minutes, and discard the supernatant.
2. Air dry the pellet at room temperature for 10 minutes.



solution at 260nm and 280nm. The $A_{260/280}$ ratio should be greater than 1.8. 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.

## 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 20mg/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 be extra careful 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.

## 3. Poor quality of DNA

DNA quality can be assessed by the measurement of its absorbance in



3. Resuspend the DNA in TE buffer or water as required.

## PROTOCOL 2 (1.1mL-3.0mL)

### Cell Preparation from Whole Blood

1. Add the blood sample to a 15mL polypropylene centrifuge tube.
2. Add 2 volumes of Reagent A.
3. Rotary mix/invert for 4 minutes at room temperature then centrifuge at 3500g for 5 minutes. Discard the supernatant without disturbing the pellet.
4. Add 2.5mL of Reagent A. Vortex/mix to resuspend the cell pellet. This should take about 1 minute.
5. Centrifuge at 3500g for 5 minutes. Discard the supernatant without disturbing the pellet.

### Cell Lysis

1. To the pellet add 350µL of Reagent B and vortex/mix to resuspend the cell pellet. This should take about 1 minute.
2. If you experience difficulty in resuspending the pellet, it may help to incubate the samples for 10 minutes at 37°C.

### Deproteinisation

1. Add 125µL of Reagent C and mix by inverting at least 7 times.
2. Add 100µL of Nucleon Resin drop-wise to the top of the sample and without further mixing, centrifuge at 3500g for 4 minutes.

### DNA Precipitation

1. Without disturbing the pellet, transfer the supernatant to a clean 15mL polypropylene centrifuge tube.
2. Add 1 volume of 100% propan-2-ol to the recovered supernatant and invert the tube several times until the DNA precipitate is visible.
3. Centrifuge at 4000g for 5 minutes to pellet the DNA then discard the supernatant.

### DNA Washing

1. Add 500µL 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 at 4000g for 2 minutes, and discard the supernatant.
2. Air dry the pellet at room temperature for 10 minutes.
3. Resuspend the DNA in TE buffer or water as required.

## PROTOCOL 3 (0.5mL-1mL)

### Cell Preparation from Whole Blood

1. Add the blood sample to a 2mL tube.
2. Add 1 volume of Reagent A.

3. Rotary mix/invert for 4 minutes at room temperature then centrifuge at 3500g for 5 minutes. Discard the supernatant without disturbing the pellet.
4. Add 1mL of Reagent A. Vortex/mix to resuspend the cell pellet. This should take about 1 minute.
5. Centrifuge at 3500g for 5 minutes. Discard the supernatant without disturbing the pellet.

### Cell Lysis

1. To the pellet add 350µL of Reagent B and vortex/mix to resuspend the cell pellet . This should take about 1 minute.
2. If you experience difficulty in resuspending the pellet, it may help to incubate the samples for 10 minutes at 37°C.

### Deproteinisation

1. Add 125µL of Reagent C and mix by inverting at least 7 times.
2. Add 100µL of Nucleon Resin drop wise to the top of the sample and without further mixing, centrifuge at 3500g for 4 minutes.

### DNA Precipitation

1. Without disturbing the pellet, transfer the supernatant to a clean 2mL tube.
2. Add 1 volume of 100% propan-2-ol to the recovered supernatant and invert the tube several times until the DNA precipitate is visible.
3. Centrifuge at 4000g for 5 minutes to pellet the DNA then discard the supernatant.

### DNA Washing

1. Add 500µL 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 at 4000g for 2 minutes, and discard the supernatant.
2. Air dry the pellet at room temperature for 10 minutes.
3. Resuspend the DNA in TE buffer or water as required.

## 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:***

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, an RNase A digestion step should be included. RNase should be made up in water and boiled for 10 minutes to inactivate the DNase. Add 15µL 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 B.

***Note 3:***

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

## 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.