GE Healthcare

# illustra Nucleon Genomic DNA Extraction Kits

## Product Booklet

Codes: RPN8501
RPN8502
RPN8509
RPN8512

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# 1. Components of the system

1. *Nucleon kits for the extraction of genomic DNA from blood and animal cell cultures (illustra™ Nucleon BACC, product code RPN8501 / RPN8502 / RPN8512) consist of:*

| Component | Product Code<br>Nucleon BACC1<br>RPN8501 | Nucleon BACC2<br>RPN8502 | Nucleon BACC3<br>RPN8512 |
|---|---|---|---|
| Reagent A | 220 ml | 420 ml | 510 ml<br>(4 x concentrate) |
| Reagent B | 18 ml | 110 ml | 110 ml |
| 5 M Sodium perchlorate | 6 ml | 26 ml | 26 ml |
| Nucleon resin | 8 ml | 16 ml | 16 ml |

2. *Nucleon kits for the extraction of genomic DNA from hard tissue (illustra Nucleon HT, product code RPN8509) consist of:*

| Component | |
|---|---|
| Reagent B | 18 ml |
| 5 M Sodium perchlorate | 6 ml |
| Nucleon resin | 8 ml |
| Proteinase K | 10 mg |

# 2. Safety warnings and precautions

**Warning: For research use only.**
**Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.**

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Note that the protocol requires the use of:
Chloroform: carcinogen, Cat 3, harmful, irritant.
Ethanol: highly flammable.
Xylene: harmful, irritant.
Conc$^{n}$ hydrochloric acid: corrosive.
Sodium hydroxide: corrosive, irritant.

Please follow the manufacturer's Safety Data Sheet relating to the safe handling and use of these reagents.

# 3. Storage and stability

**Storage**

Store at room temperature, 15–30˚C. Proteinase K should be stored at 2–8˚C.

**Stability**

The Nucleon kit components are stable for up to 18 months, 3 months once opened, when stored under the recommended conditions. Performance is consistent when stored under the recommended conditions using the recommended procedures.

**Expiry**

For expiry date please refer to outer packaging label.

# 4. Description

Nucleon™ resin contained within the Nucleon genomic extraction kits was first developed by Professor Brian Caddy at Strathclyde University, for use in extracting DNA from difficult forensic samples. The protocols and Nucleon resin have been further developed by scientists within Tepnel Life Sciences and by a number of academic collaborators.

The systems supplied by GE Healthcare are designed to give high yields of pure DNA from blood, cultured cells, hard tissue or paraffin sections. The procedures have been optimized to give maximum recoveries of high molecular weight DNA using a low shearing protocol. The unique system employing the Nucleon resin removes protein effectively without the use of phenol.

Typical yields and purities are shown in Table 1. The size of the DNA recovered from these tissues ranges from 23 to 250 kbp, as determined by pulse field gel electrophoresis. The combination of purity and high molecular weight makes the recovered DNA suitable for a variety of molecular biology applications.

Table 1: Typical yields and purities.

| | Sample | Yield<br>µg DNA/mg tissue | mean purity<br>A 260/280 |
|---|---|---|---|
| Nucleon | | | |
| BACC1/2/3 | Blood (10 ml) | 370–440 | 1.8 |
| | HeLa cells ($10^6$ cells) | 12 | 1.8 |
| Nucleon HT | Mouse tail per cm* | 70–200 | 1.9 |
| | Xiphisternum | 1.6–1.9 | 1.6 |

* yield will vary depending on the section of tail used. 1 cm of tail equals approximately 60 mg of tissue.

**Nucleon BACC for blood and cell cultures.**
(see flow diagram on page 8)

Nucleon blood and cell culture (BACC) extraction kits are available in 3 formats. Please note that the BACC2 kit is supplied with sufficient volume of the non-proprietory Reagent A for 10 preparations from 10 ml of blood or $3 \times 10^6$ to $1 \times 10^7$ cells.
Reagent A preparation can be found in the additional information section of the protocol booklet.

**illustra Nucleon BACC1**
for 50 preparations from 1 ml of whole blood or 1 to $3 \times 10^6$ cultured cells.

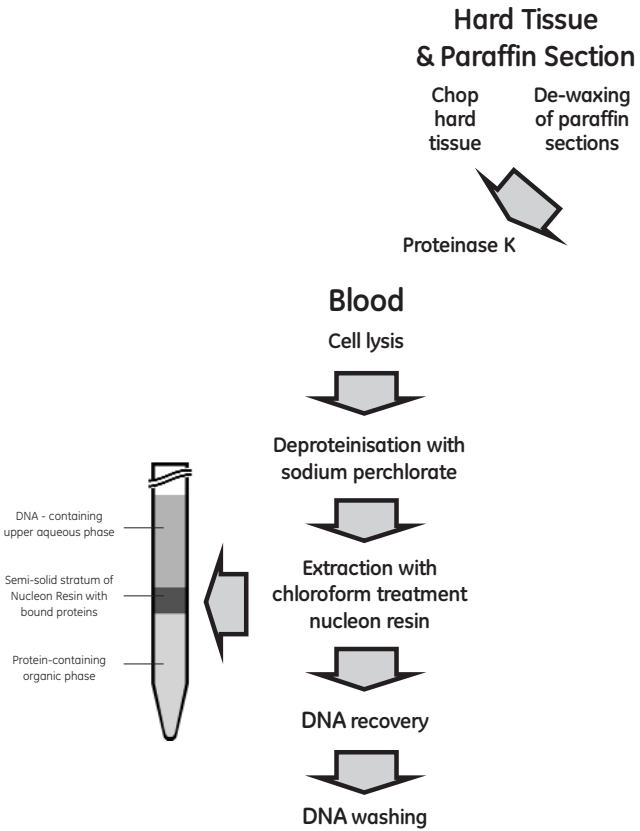
**illustra Nucleon BACC2**
for 50 preparations from 10 ml of whole blood or 3 to $10^6$ to $1 \times 10^7$ cultured cells.

**illustra Nucleon BACC3**
for 50 preparations from 10 ml of whole blood.

**illustra Nucleon HT for hard tissue and paraffin sections**
(see flow diagram on page 8)

Nucleon HT protocols have been developed for those tissues which do not homogenize easily in lysis buffer and require digestion with proteinase K. The kit has sufficient reagents for 50, 25 mg preparations or 50 paraffin sections.

# 5. Flow diagram showing the principle steps in the nucleon protocol



# 6. Critical parameters

- It is strongly advised that an aseptic technique be used in conjunction with the described protocol, particularly when handling Reagent A. Once opened store at 2–8˚C.
- Proteinase K is supplied as a powder which should be stored at 2–8˚C. Before use dissolve in 1 ml of sterile water. Store the solution at 2-8˚C for up to three months.
- Reagent A in the Nucleon BACC3 kits is supplied as a 4x concentrate. Prior to first use it should be diluted four fold with deionized water and autoclaved in suitable aliquots.
- It has been reported that heparin can bind to DNA during extraction. If present in the final DNA solution it can cause interference with PCR* techniques (1). The effect of heparin may be counteracted in several ways (2,3).

# 7. Additional equipment and solutions

## 7.1 Equipment

- Microcentrifuge
- Bench top centrifuge
- 1.5 ml polypropylene microcentrifugation tubes
- 12 mm diameter polypropylene screw capped tubes
- Assorted range of high precision pipettes
- Water bath
- Rotary mixer
- Homogenization tube

## 7.2 Solutions

- Chloroform, AnalaR™ grade or similar
- Ethanol, AnalaR grade or similar
- Xylene, AnalaR grade or similar
- RNase solution, 50 µg/ml in sterile water
- Dry ice
- TE buffer

1.21 g Trizma™ base
0.372 g Ethylenediaminetetra-acetic acid, sodium salt
Add approximately 800 ml of distilled water. Mix to dissolve. Adjust to pH 8.0 with concentrated hydrochloric acid. Make up to a final volume of 1000 ml. Autoclave in suitable aliquots.
Store up to 3 months at room temperature.

# 8. Protocols

## 8.1 BACC1 for small blood volumes (50 µl-1.0 ml) and 1 x $10^6$ to 3 x $10^6$ cultured cells.

The protocol requires the use of cold 70%(v/v) and absolute ethanol.

| Protocol | Notes |
|---|---|
| **Cell Preparation** | |
| **1.** Collect the blood (50 µl–1.0 ml) in sodium EDTA tubes.<br>Or<br>Collect the cultured cells by centrifuging at 600 g for 5 minutes at 4˚C. Discard the supernatant without disturbing the pellet. | **1.** In order to minimize damage to DNA in collected blood samples, blood which is stored at 4˚C should be extracted within 24 hours of collection. Heparinized and citrated blood is also suitable. Heparin may interfere with subsequent procedures, see critical parameters page 9. Harvested cells from buccal swabs/spatulas in appropriate media for example isotonic saline, sucrose etc. and buffy coat preparations can be processed using the BACC protocols. |
| **Cell Lysis** | |
| **2.** Using an aseptic procedure add 4 times the volume of Reagent A to the blood sample. Rotary mix for 4 minutes at room temperature. Centrifuge at 1300 g for 5 | **2.** Due to the high sucrose content, Reagent A could be contaminated if aseptic technique is not used when dispensing. |

| Protocol | Notes |
|---|---|
| minutes. Discard the supernatant.<br>Or<br>Resuspend cells in 1.0 ml Reagent A and leave on ice for 5 minutes. Centrifuge at 1300 g for 5 minutes and discard the supernatant. | |
| **3.** To the pellet add 350 µl of Reagent B. Vortex briefly to resuspend the pellet. Transfer the suspension to a 1.5 ml microcentrifuge tube. | **3.** Ensure that Reagent B is completely dissolved. This may be achieved using gentle heat. Incubating the samples at 37˚C for 10 minutes can also help to resuspend the pellets. |
| **RNase treatment (optional)** | |
| **4.** Following resuspension and transfer to a clean tube add 2.5 µl of a 50 µg/ml RNase solution. Incubate the tube in a water bath at 37˚C for 30 minutes. | **4.** Extracted DNA from cultured cells may contain small amounts of RNA. If RNA free DNA is required, an RNase digestion step should be included. RNase should be made up in water and boiled for 10 minutes to inactivate any contaminating DNase. |
| **Deproteinisation** | |
| **5.** Add 100 µl of sodium perchlorate solution. Mix by hand, inverting the capped tube at least 7 times. | |

| Protocol | Notes |
|---|---|
| **DNA extraction** | |
| **6.** Add 600 µl of chloroform (not supplied). Mix by hand, inverting the capped tube at least 7 times. | |
| **7.** Without remixing the phases add 150 µl of Nucleon resin. Centrifuge at 350 g for 1 minute. | **7.** A spin speed of 350 g corresponds to 2000 rpm in an Eppendorf™ 5415 Microfuge. Check the manual accompanying your machine or refer to the additional information section page 36. Speeds higher that those given may cause the resin to spin to the bottom of the tube. |
| **DNA precipitation** | |
| **8.** Without disturbing the Nucleon resin layer (brown in color), transfer the upper phase (approximately 450 µl) to a clean 1.5 ml Microcentrifuge tube. | **8.** The resin layer should not be disturbed in order to minimize contamination from the protein interface. A white protein layer may also be associated with the resin. This layer must not be disturbed. If any resin has been carried over, centrifuge briefly at a minimum of 1300 g to pellet the resin, and then transfer to a clean tube. The resin, if carried over, will not interfere with subsequent processing. |

**9.** Add 2 volumes (900 µl) of cold absolute ethanol. Mix by inversion until the precipitate appears.

**DNA washing**

**10.** Centrifuge at top speed (minimum 4000 g) for 5 minutes to pellet the DNA. Discard the supernatant.

**11.** Add 1 ml cold 70% (v/v) ethanol, mix several times by inversion. Re-centrifuge and discard the supernatant. This step can be repeated if necessary.

**12.** 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. 50–250 µl). The DNA should re-dissolve within 2 hours when using a rotary mixer.

## 8.2 BACC2/3 for 3 - 10 ml blood and 3 x $10^6$ to 1 x $10^7$ culture cells.

**Note:** Reagent A in the BACC3 kit is supplied as a 4x concentrate. Prior to first use it should be diluted four fold with deionized water and autoclaved, at 121˚C 15 psi for 15 minutes, in suitable aliquots. The protocol requires the use of cold 70%(v/v) and absolute ethanol.

| Protocol | Notes |
|---|---|
| **Cell Preparation** | |
| **1.** Collect the blood in sodium EDTA tubes.<br>Or<br>Collect the cultured cells by centrifuging at 600 g for 5 minutes at 4˚C. Discard the supernatant without disturbing the pellet. | **1.** In order to minimize damage to DNA in collected blood samples, blood which is stored at 4˚C should be extracted within 24 hours of collection. Heparinized and citrated blood is also suitable. Heparin may interfere with subsequent procedures, see critical parameters page 9. Harvested cells from Buccal swabs/spatulas in appropriate media for example isotonic saline, sucrose etc. and buffy coat preparations can be processed using the BACC protocols. |
| **Cell Lysis** | |
| **2.** Using an aseptic procedure add 4 times the volume of Reagent A to the blood sample. Rotary mix for 4 minutes at room | **2.** Due to the high sucrose content, Reagent A could be contaminated if aseptic technique is not used when dispensing. |

| Protocol | Notes |
|---|---|
| temperature. Centrifuge at 1300 g for 5 minutes. Discard the supernatant. Or Resuspend cells in 1.0 ml Reagent A and leave on ice for 5 minutes. Centrifuge at 1300 g for 5 minutes and discard the supernatant. | |
| **3.** To the pellet add 2 ml of Reagent B. Vortex briefly to resuspend the pellet. Transfer the suspension to a 15 ml screw capped propropylene centrifuge tube. | **3.** Ensure that Reagent B is completely dissolved. This may be achieved using gentle heat. The internal diameter of the tube should not exceed 12 mm. Incubating the samples at 37˚C for 10 minutes can also help to resuspend the pellets. |
| **RNase treatment (optional)** | |
| **4.** Following resuspension and transfer to a clean tube add 15 µl of a 50 µg/ml RNase solution. Incubate the tube in a water bath at 37˚C for 30 minutes. | **4.** Extracted DNA from cultured cells may contain small amounts of RNA. If RNA free DNA is required, an RNase digestion step should be included. RNase should be made up in water and boiled for 10 minutes to inactivate any contaminating DNase. |
| **Deproteinisation** | |
| **5.** Add 500 µl of sodium | |

| Protocol | Notes |
|---|---|
| perchlorate solution. Mix by hand, inverting the capped tube at least 7 times. | |
| **DNA extraction** | |
| **6.** Add 2 ml of chloroform (not supplied). Mix by hand, inverting the capped tube at least 7 times. | |
| **7.** Without remixing the phases add 300 µl of Nucleon resin. Centrifuge at 1300 g for 3 minutes. | **7.** A spin speed of 1300 g corresponds to 2400 rpm in a centrifuge with a swing-out bucket rotor radius 190 mm. Check the manual accompanying your machine or refer to the additional information section page 36. Speeds higher than those given may cause the resin to spin to the bottom of the tube. |
| **DNA precipitation** | |
| **8.** Holding the tube vertically without disturbing the Nucleon resin layer (brown in color), transfer the upper phase (approximately 2.5 ml) to a clean tube of minimum volume 7.5 ml. | **8.** The resin layer should not be disturbed in order to minimize contamination from the protein interface. A white protein layer may also be associated with the resin and this must be avoided. If any resin has been carried over, centrifuge briefly at a |

| Protocol | Notes |
| --- | --- |
| | minimum of 1300 g to pellet the resin, and then transfer to a clean tube. The resin, if carried over, will not interfere with subsuquent processing. |
| **9.** Add 2 volumes of cold absolute ethanol. Mix by inversion until the precipitate appears. | **9.** Precipitated DNA may be hooked out at this stage 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. |
| **DNA washing** | |
| **10.** Centrifuge at top speed (minimum 4000 g) for 5 minutes to pellet the DNA. Discard the supernatant. | |
| **11.** Add 2 ml cold 70% (v/v) ethanol, mix several times by inversion. Re-centrifuge and discard the supernatant. This step can be repeated if necessary. | |
| **12.** 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. 1.0–2.0 ml). | |

The DNA should re-dissolve within 2 hours when using a rotary mixer.

## 8.3 Nucleon HT for DNA extraction from hard tissue

Note: Proteinase K is supplied as a powder which should be stored at 2–8˚C. Before use dissolve in 1 ml of sterile water. Store the solution at 2–8˚C for up to three months.

The protocol requires the use of cold 70%(v/v) and absolute ethanol.

| Protocol | Notes |
|---|---|
| **Tissue preparation and lysis** | |
| **1.** Grind 25 mg of tissue on dry ice or in liquid nitrogen to a fine powder and transfer to a 1.5 ml microcentrifuge tube. | **1.** For mouse tails it may be necessary to grind the tissue with pestle and mortar after finely chopping. |
| **2.** Add 0.35 ml of Reagent B. | **2.** Ensure that Reagent B is completely dissolved. This may be achieved using gentle heat. |
| **RNase treatment (optional)** | |
| **3.** Add RNase solution to a final concentration of 400 ng/ml. Incubate the tube in a water bath at 37˚C for 30 minutes. | **3.** Extracted DNA from tissue may contain small amounts of RNA. If RNA-free DNA is required, an RNase digestion step should be included. RNase should be made up in water and boiled for 10 minutes to inactivate any contaminating DNase. |
| **4.** Add 18 µl of the proteinase K solution and incubate at 50˚C for at least 3 hours (or overnight). | |
| **5.** Centrifuge at 2000 g for | |

| Protocol | Notes |
| --- | --- |
| 5 minutes. Remove the supernatant and transfer to a clean tube. | |
| **Deproteinisation** | |
| **6.** Add 100 µl of sodium perchlorate solution. Mix by hand, inverting the capped tube at least 7 times. | |
| **DNA extraction** | |
| **7.** Add 600 µl of chloroform (not supplied). Mix by hand, inverting the capped tube at least 7 times. | |
| **8.** Add 150 µl of Nucleon resin and without remixing the phases centrifuge at 350 g for 1 minute. | **8.** A speed of 350 g corresponds to 2000 rpm in an Eppendorf 5415 Microfuge. Check the manual accompanying your machine or refer to the additional information section page 36. |
| **DNA precipation** | |
| **9.** Without disturbing the Nucleon resin layer (brown in color), transfer the upper phase to a clean tube. | **9.** The resin layer should not be disturbed in order to minimize contamination from the protein interface. A white protein layer may also be associated with the resin. This layer must be avoided. The resin, if carried over, will |

| Protocol | Notes |
| --- | --- |
| | not interfere with subsequent processing. If any resin has been carried over, centrifuge briefly at a minimum of 1300 g to pellet the resin, and then transfer to a clean tube. |
| **DNA washing** | |
| **10.** Add 2 volumes of cold absolute ethanol. Mix by inversion until the precipitate appears. | **10.** Precipitated DNA may be hooked out at this stage 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. |
| **11.** Centrifuge at top speed (minimum 4000 g) for 5 minutes to pellet the DNA. Discard the supernatant. | |
| **12.** Add 1.0 ml cold 70% (v/v) ethanol, mix several times by inversion. Re-centrifuge and discard the supernatant. This step can be repeated if necessary. | |
| **13.** 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. The DNA should re-dissolve within 2 hours when using a rotary mixer.

## 8.4 Nucleon HT for DNA extraction from paraffin sections

Note: Proteinase K is supplied as a powder which should be stored at 2–8˚C. Before use dissolve in 1 ml of sterile water. Store the solution at 2–8˚C for up to three months.

The protocol requires the use of cold 70%(v/v) and absolute ethanol.

| Protocol | Notes |
|---|---|
| **Preparation of paraffin sections** | |
| **1.** Take one 20–30 micron section of tissue and place in a 1.5 ml microcentrifuge tube. | |
| **2.** Cover the section in xylene. Incubate at 37˚C for 20 minutes. Centrifuge at 1300 g for 5 minutes and remove the xylene. | **2.** Check the manual accompanying your machine or refer to the additional information section page 36. |
| **3.** Incubate in xylene at room temperature for 2 minutes. Centrifuge at maximum speed for 5 minutes and remove all the xylene. | |
| **4.** Rehydrate the section by washing consecutively in 100% ethanol, 75(v/v)% ethanol, 50(v/v)% ethanol, 25(v/v)% ethanol and finally water. Centrifuge at maximum speed for 1–3 minutes between each wash. | **4.** Care should be taken at the 25%(v/v) ethanol and water stages as the material can become loose and difficult to pellet. |
| **5.** Remove the water from the | **5.** Ensure that Reagent B is |

| Protocol | Notes |
| --- | --- |
| pellet and add 0.35 ml of Reagent B. | completely dissolved. This may be achieved using gentle heat. |
| **6.** Add 18 µl of the proteinase K solution and incubate at 55˚C overnight for maximum yield. | **6.** A three hour incubation should provide an adequate yield. |
| **Deproteinisation** | |
| **7.** To the 350 µl lysate add 100 µl of sodium perchlorate solution. Mix by hand, inverting the capped tube at least 7 times. | |
| **DNA extraction** | |
| **8.** Add 600 µl of chloroform (not supplied), Mix by hand, inverting the capped tube at least 7 times. | |
| **9.** Add 150 µl of Nucleon resin and without re-mixing the phases centrifuge at 350 g for 1 minute. | **9.** A speed of 350 g corresponds to 2000 rpm in an Eppendorf 5415 Microfuge. Check the manual accompanying your machine or refer to the additional information section page 36. |
| **DNA precipation** | |
| **10.** Without disturbing the Nucleon resin layer (brown in color), transfer the upper phase to a clean tube. | **10.** The resin layer should not be disturbed in order to minimize contamination from the protein interface. A white protein layer may also be |

| Protocol | Notes |
| --- | --- |
| | associated with the resin. This layer must be avoided. The resin, if carried over, will not interfere with subsequent processing. If any resin has been carried over, centrifuge briefly at a minimum of 1300 g to pellet the resin, and then transfer to a clean tube. |
| **11.** Add 2 volumes of cold absolute ethanol. Mix by inversion and leave at -20˚C for 1–2 hours to precipitate the DNA. | **11.** 1 µl of 20 mg/ml glycogen solution may be added as a carrier if required. |
| **DNA washing** | |
| **12.** Centrifuge at top speed for 15 minutes to pellet the DNA. Discard the supernatant. | |
| **13.** Add 1.0 ml cold 70% (v/v) ethanol, mix several times by inversion. Re-centrifuge and discard the supernatant. This step can be repeated if necessary. | |
| **14.** 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. 100 µl). The DNA should re-dissolve within 2 hours when using a rotary mixer.

# 9. Trouble shooting guide

| Problem | Possible cause | Remedy |
|---|---|---|
| **1. Cell preparation for whole blood (BACC only).** Incomplete lysis of red blood cells resulting in a white cell nuclei pellets contaminated with red cells after treatment with Reagent A and subsequent centrifugation. | **1.** Exceptionally high red cells counts in the blood sample. Or White cell clumping around red cells thereby preventing red cell lysis. | **1.** Repeat the Reagent A treatment as per the protocol using 1 ml Reagent A. |
| **2. Cell preparation for whole blood (BACC only).** White cell nuclei pellet has a hint of red coloration. | **2.** This may occur if the blood sample is not as high quality as normal for example as a result of the collection tube not being mixed or stored appropriately. | **2.** In most cases this coloration will disappear upon continuation of the protocol to the point of DNA washing. There should be no effect upon the suitability of the extracted DNA for further applications. If this is not the case, repeat the Reagent A treatment as per the protocol. |

| Problem | Possible cause | Remedy |
|---|---|---|
| **3. Cell preparation for whole blood (BACC only).** White cell nuclei pellet fails to form or be retained at the bottom of the tube. | **3.1** Insufficient centrifugal force. | **3.1** Check the calculation of g from rpm for your rotor using the formula on page 36 or from the rotor manufacturers manual. If the g calculation is correct, spin for longer until the pellet forms. |
| | **3.2** When the sample is small (e.g. <5 ml blood), the pellet may be small and difficult to visualize. | **3.2** Do not be concerned. Proceed with the protocol as normal. |
| | **3.3** Pellets are more likely to dislodge from round-bottomed tubes than with conical-bottomed tubes. | **3.3** Use conical-bottomed tubes or be extra careful when decanting the supernatant. |
| **4. Cell lysis** Incomplete cell lysis. | **4.1** Detergent in Reagent B has come out of solution. | **4.1** Re-dissolve the detergent by heating to 35˚C and agitating. |

| Problem | Possible cause | Remedy |
|---|---|---|
| | **4.2** Too many cells in the sample. | **4.2** 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, sodium perchlorate and chloroform are used to prevent overloading of the system. |
| | **4.3** Pelleted material "clumps" often as a result of 4.2. above. | **4.3** If "clumping" is the problem, ensure that the pellet is fully re suspended to homogeneity in Reagent B by vigorous vortexing or extended incubation at 37˚C or room temperature. |
| **5. Cell lysis** Incomplete lysis of pellet stored in DMSO or isolated from a Ficoll gradient (for | **5.** Contamination of pellet with DMSO or Ficoll. | **5.** Wash pellet in phosphate buffered saline prior to adding Reagent B. |

| Problem | Possible cause | Remedy |
|---|---|---|
| example Buffy Coat preparations). | | |
| **6. DNA Extraction** The brown Nucleon resin fails to form a layer at the interface between the chloroform and aqueous phase. | **6.1** The system is overloaded due to the presence of too many DNA containing cells. The resin does not clear the upper phase due to high viscosity caused by high concentrations of DNA. | **6.1** Reduce the sample size to fall within the recommended range for the protocol in use. |
| | **6.2** The centrifugation speed is incorrect. If too high, the resin may pellet at the bottom of the tube. | **6.2** Check the calculation of g from rpm using the formula on page 36 or from the rotor manufacturers manual. Please note that if a small amount of the resin does spin to the bottom of the tube (particularly with the small volume protocols), this will not affect the quality of the DNA preparation. |

| Problem | Possible cause | Remedy |
|---|---|---|
| | **6.3** Tubes with inappropriate dimensions are being used. | **6.3** The protocol has been optimized using tubes with internal diameters which ensure optimal volumes of Nucleon resin to enable adequate protein binding and barrier formation at the interface. |
| **7. DNA precipitation** Some Nucleon resin is carried over into the DNA pellet which appears brown/red in color. | **7.** Insufficient care has been taken when removing the upper phase or the centrifugation after this manipulation has not been performed. | **7.** Follow the protocol as stated with adequate care. |
| **8. DNA precipitation** A non-DNA insoluble white precipitate forms on the addition of ethanol. | **8.** An excess of ethanol has been added. | **8.** Ensure that the recommended volumes of ethanol are not exceeded. |
| **9. DNA washing** The DNA pellet will not re-dissolve or re-dissolves slowly. | **9.1** The DNA pellet has been excessively dried. | **9.1** Follow the recommended drying conditions in the protocol. |
| | **9.2** The DNA is not pure. | **9.2** Please see 11 below. |

| Problem | Possible cause | Remedy |
|---|---|---|
| | **9.3** The white precipitate discussed in 8 above is being mistaken for DNA. | **9.3** Please see 8 above. |
| **10. DNA Yield**<br>Low Yield of DNA. | **10.1** Too few nucleated cells present in the starting sample. | **10.1** Check cell count prior to cell lysis step to ensure that the sample falls within the recommended range.<br>If a low yield is expected due to an extremely small sample size, recovery may be enhanced by adding a carrier DNA (e.g. denatured herring sperm DNA) or glycogen (1 µl of 20 mg/ml glycogen per 600 µl ethanol). |
| | **10.2** Too many nucleated cells present in the starting sample. If this is the case, incomplete lysis may occur (see 4 above). | **10.2** System is overloaded, (see 4 above). |

| Problem | Possible cause | Remedy |
|---|---|---|
| | **10.3** Incomplete lysis (see 4 above). | **10.3** See 4 above. |
| | **10.4** Poor recovery of DNA when precipitated with ethanol. | **10.4.1** Check the calibration of the UV spectrophotometer using a standard DNA solution. |
| | | **10.4.2** See 10.1 above |
| | **10.5** Inaccurate UV measurement. | **10.5** Check the calibration of the UV spectrophotometer using a standard DNA solution |
| | **10.6** Loss of DNA-containing pellet after discarding Reagent A. | **10.6** Take care when removing Reagent A. If in doubt, use a pipette and carefully drain the remaining Reagent A onto a tissue. |
| **11. Poor quality DNA** The A260/280 ratio is too high or too low. The ratio should fall in the range 1.7–1.9. | **11.1** Low ratios usually indicate protein contamination. | **11.1** Avoid using sample sizes which exceed the range for the protocol being used. |

| Problem | Possible cause | Remedy |
|---|---|---|
| | **11.2** The DNA is not thoroughly re-dissolved after precipitation. | **11.2** Ensure that the DNA is fully dissolved after washing. After DNA pellet resuspension, leave the samples overnight at 4˚C. |

# 10. Additional information

## 10.1 Calculation of centrifugal force

To ensure that Nucleon protocols are universally applicable to all centrifuges, centrifugal force is expressed as g rather than rpm values. To convert rpm to g please refer to the rotor manufacturers manual. If this is not available use the formula illustrated below.

$g = 1.12r\ (rpm/1000)^2$
$rpm = 1000\sqrt{(g/1.12r)}$
r = maximum radius of the rotor in mm

## 10.2 Additional reagent preparation

**Reagent A for illustra Nucleon BACC2 kits**

10 mM Tris-HC1, 320 M sucrose, 5 mM $MgCl_2$,
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.

# 11. References


1. BEUTLER, E. *et al., Bio Techniques*, **9** (2), p166, 1990.
2. POLI, F. *et al., PCR Methods and Applications*, **2,** pp356-358, 1993.
3. TSAI, M. *et al., American Journal of Pathology*, **146** (2), pp335-343, 1995.

# 12. DNA extraction products

**illustra Nucleon HT,**
for hard tissue, and paraffin sections,
50 preparations of up to 25 mg per prep. **RPN8509**

**illustra Nucleon PhytoPure™,**
for plant and fungal DNA extraction kit,
50 preparations of 0.1 g. **RPN8510**

**illustra Nucleon PhytoPure,**
for plant and fungal DNA extraction kit,
50 preparations of 1.0 g. **RPN8511**

**illustra Nucleon BACC1,**
for 50 preparations of 1 ml whole blood or
cultured cells (1 to 3 x $10^6$). **RPN8501**

**illustra Nucleon BACC2,**
for 50 preparations of 10 ml of whole blood or
cultured cells (3 x $10^6$ to 1 x $10^7$). **RPN8502**

**illustra Nucleon BACC3,**
for 50 preparations of 10 ml of whole blood. **RPN8512**

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http://www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA
UK

**GE Healthcare offices:**
GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84
Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5 D-79111
Freiburg
Germany

GE Healthcare Bio-Sciences
Corp
800 Centennial Avenue
P.O. Box 1327
Piscatway
NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1
Hyakunincho Shinjuku-ku
Tokyo 169-0073
Japan

For contact information for your local office,
please visit: www.gelifesciences.com/contact

GE Healthcare UK Limited.
Amersham Place,
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

http://www.gehealthcare.com/lifesciences

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