

Whole Blood DNA
Extraction Protocol with
MACHERY-NAGEL
NucleoSpin® 96 Blood kit
Cat# 740 664 (8 Well Strip System)
Cat# 740 665.X (96 Well Plate)

X-tractor Gene™



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Protocol Description

Introduction

The Whole Blood DNA Extraction Protocol described here is designed for walk-away automated preparation of DNA from a variety of blood-derived samples (for example freshly collected, frozen, buffy coat fraction, cultured cells, etc). Final extracted DNA is of high quality and suitable for a wide variety of downstream applications.

Purification kits are available in 96-well plates or in a flexible 8-well strip format.

With the **NucleoSpin® 8/96 Blood** method, genomic DNA is isolated after lysis achieved by incubation of whole blood in a solution containing chaotropic ions in the presence of Proteinase K at room temperature. Appropriate conditions for binding of DNA to the glass fibre membrane in the **NucleoSpin® Blood Binding Module** are created by addition of ethanolic binding buffer to the lysate. The binding process is reversible and specific to nucleic acids. Contaminants are removed by three wash steps with ethanolic buffers. Finally, pure genomic DNA is eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Sample Volume

For trouble-free operation, samples must be as consistent as possible; a particulate and clot-free liquid is ideal. In addition, processing too much sample will cause the glass fibre matrix to block and the extraction to fail. The protocol is suitable for fresh or frozen blood. Blood treated either with EDTA, citrate, heparin or CPDA can be used. If leukocyte rich materials like buffy coat or animal blood are used, apply smaller volumes or dilute samples with sterile PBS buffer

Using this protocol with MACHEREY-NAGEL reagents, silica plates and consumables a *maximum* of 200 µL of whole blood may be processed. Each sample may contain up to 1×10^6 nucleated cells and will provide yields of up to 7 µg of DNA.

The maximum amount of sample and the yield obtained depends on the species source and white blood cell count. Please note that exceeding the 200 µL sample maximum cannot be supported. Note also the high white-cell count of some sample types will cause even 200 µL samples to block the glass fibre matrix. It is up to the user to empirically determine the maximum sample size for their particular sample type.

Processing time

Total time required to complete the DNA extraction procedure depends on the number of samples processed. Typically, a single column of 8 samples requires 45 minutes to complete. Each additional column of 8 samples adds a further 5 minutes to the total processing time. Thus a full 96-well plate requires about 95 minutes to complete.

IMPORTANT

Wear gloves and a laboratory coat throughout the procedure.

Take care to avoid cross-contamination of samples and reagents.

Make sure reagent tubs, tubes and plates are clearly labelled and clean.

Protocol Validation

Verification Testing

Whole Blood protocol has been functionally developed on the Corbett Robotics X-tractor Gene™ Automated Extraction System using NucleoSpin® 96 Blood kit provided reagents and consumables. Typical results are shown below. Actual results will vary depending upon sample age, quality, type, species of subject and nucleated cell count.

Samples

Whole human blood was collected in an EDTA Vacutainers. Blood from different donors was pooled and stored either at 4°C or at -20°C. 96 samples total (whole 96-well plate) were processed, comprising 96 whole blood samples (48 samples from blood stored at 4°C, 48 from blood stored at -20°C). The maximum whole blood sample size (200 µL) was processed per well.

Cross Contamination Test

To maximise the detection of any potential contamination event, 48 positive and 48 no template controls (NTCs) were arranged in alternating wells (in a “checkerboard” pattern. Fig. 1). PCR analysis was done on all samples targeting the β-actin gene in a 40 cycle PCR. No amplification of NTCs was observed indicating no cross-contamination.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Sample(+)

NTC(-)

Fig.1 Illustration of the ‘Checkerboard Pattern’ utilized for the cross contamination analysis test.

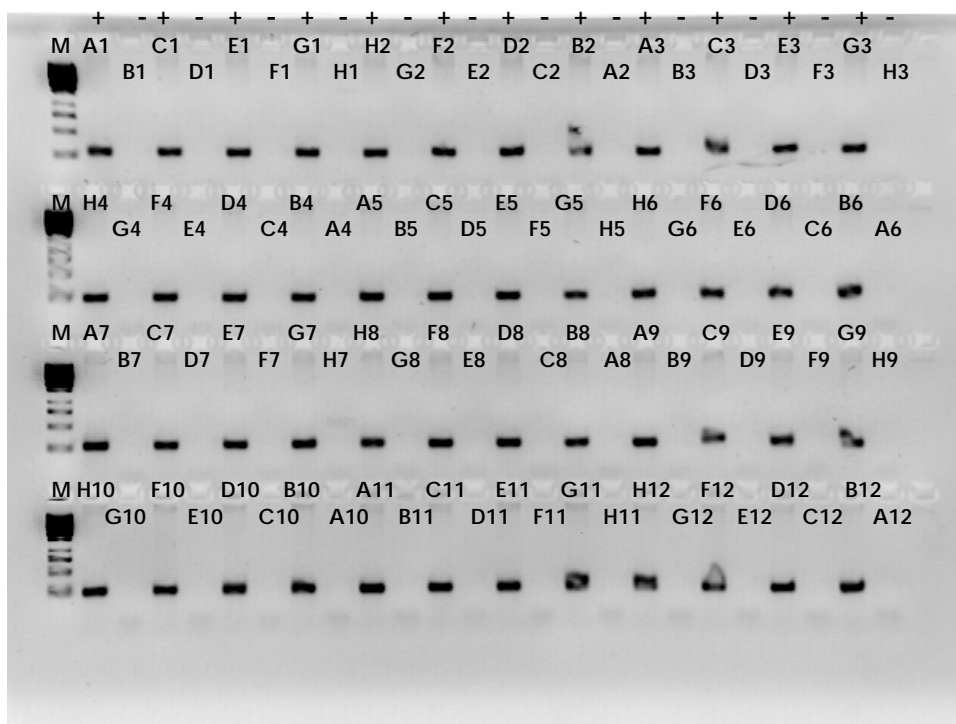


Fig. 2 Cross contamination analysis. Blood samples and water (NTC) were arrayed in adjacent wells of the sample block. After purification 2 μ L eluted DNA of each well was analysed by PCR using β -actin primers over 40 cycles. 10 μ L of PCR reaction was analysed by agarose gel electrophoresis. No PCR product was detected from NTC samples indicating that there was no cross contamination. Expected PCR fragment size of samples is 200 bp.

Yield Reproducibility

Yield, purity and reproducibility from all samples were measured by UV spectroscopy (see next page). Furthermore aliquots of the purified DNA was analysed by real time PCR using SYBR green™ detection. Results are summarized as follows:

Reproducibility of DNA yield from all 24 randomly selected samples was measured as threshold cycle (C_T) values from real-time PCR analysis. Sample DNA aliquots were amplified in a LightCycler™ instrument system using SYBR green™ chemistry. Results (Fig. 1) are summarised as follows:

Maximum C_T	22.95
Minimum C_T	21.44
Average C_T	22.12
C_T Std. Deviation	0.37

PCR Inhibitor Test

Reproducibility of real-time PCR C_T values and end-point product yield analysis showed no evidence of PCR inhibitors in any of the extracted DNA samples.

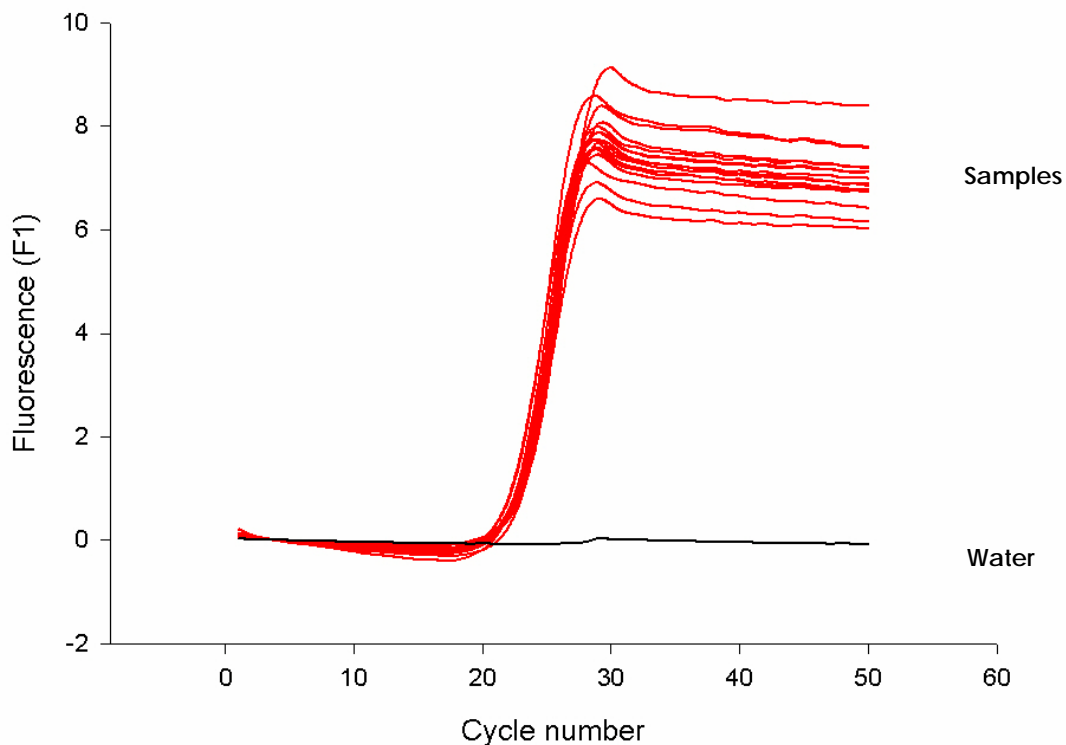


Fig. 3 Real-time PCR analysis of DNA extracted from whole human blood. 16 randomly selected samples were analysed on a LightCycler™ real-time PCR instrument system using β -actin primers over 40 PCR cycles. Results show strong and reproducible amplification, and no evidence of PCR inhibitors.

Spectrophotometer Analysis

All 96 samples were analysed for yield and purity (Fig. 3, Fig. 4). Results are summarized below.

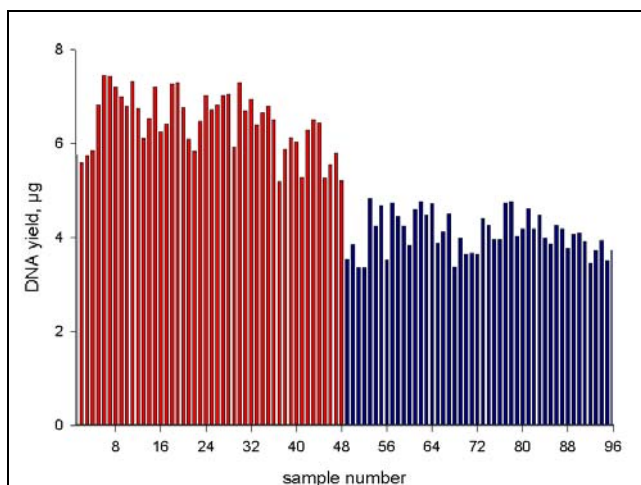


Fig. 3 Example DNA yield ($\mu\text{g}/200 \mu\text{L}$ of human blood) sample 1-48 fresh blood, sample 49-96 blood stored at -20°C

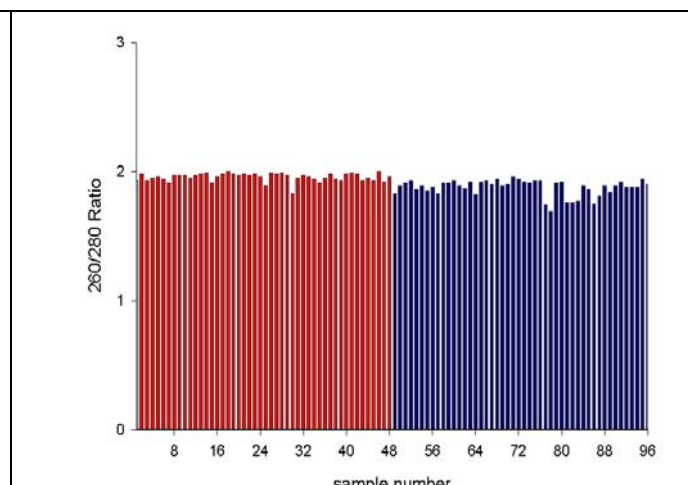


Fig 4. Example 260/280 ratios sample 1-48 fresh blood, sample 49-96 blood stored at -20°C

Samples 1-48 (4°C)			
	Yield	Concentration	260/280 Ratio
Maximum	7.5 µg	47 ng/µL	1.99
Minimum	5.2 µg	33 ng/µL	1.83
Average	6.4 µg	40 ng/µL	1.96
Standard Deviation	0.6 µg	3.7 ng/µL	0.03

Samples 49-96 (-20°C)			
	Yield	Concentration	260/280 Ratio
Maximum	4.8 µg	30 ng/µL	1.96
Minimum	3.4 µg	26 ng/µL	1.70
Average	4.1 µg	26 ng/µL	1.88
Standard Deviation	0.4 µg	2.5 ng/µL	0.06

Agarose Gel Electrophoresis Analysis

Agarose gel electrophoresis of raw extracted product (Fig. 5) showed reproducible yields of high quality, intact high molecular weight DNA (>20 kb).

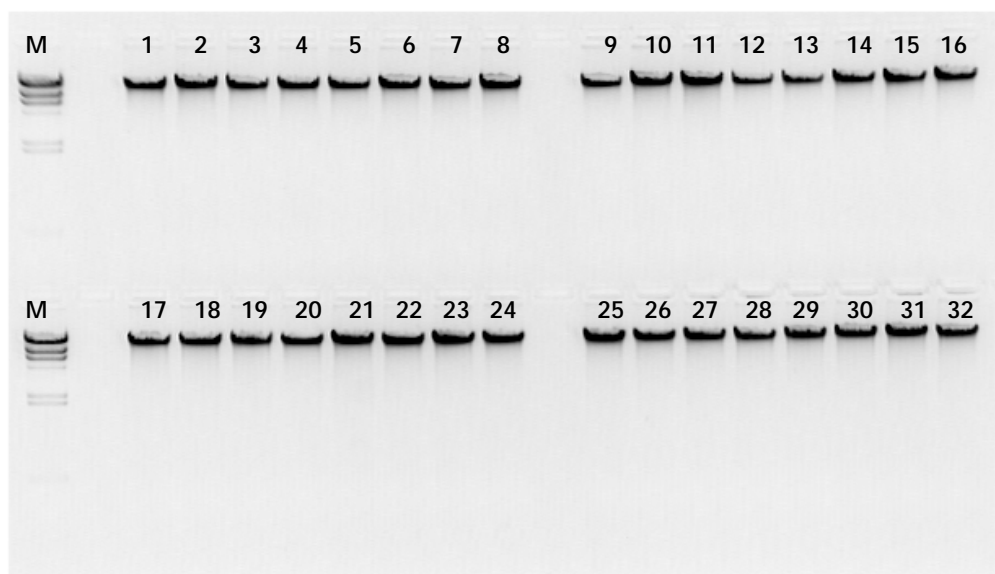


Fig. 5 Agarose gel electrophoresis results for human whole blood DNA extractions.

Sample wells contain 10 µL aliquots from the 160 µL total recovered eluate of DNA extract obtained from 200 µL of human whole blood.

Electrophoresis conditions: 1.0% agarose gel, 1× TAE running buffer, ethidium bromide stain.

M: Marker λHindIII (fragment sizes: 23.1 kbp, 9.4 kbp, 6.5 kbp, 4.3 kbp, 2.3 kbp, 2.0 kbp, 0.5 kbp),

1-32: DNA isolated from blood samples stored at -20°C.

Reagents and Consumables Required

Item	Requirement for a Full 96-Well or 48 Well Extraction	Part No. or Part of (MN)	Supplier	
Consumables				
NucleoSpin® Blood Binding Plate ¹ (for 96 well plate extractions) ¹	1 Plate	740 655.1 ²	MN	
NucleoSpin® Starter Set A ³ (Accessory for 8 Strip system)	1 Column Holder	740 682		
NucleoSpin® Blood Binding Strips (for 8 Strip extractions) ¹	6 strips	740 664 ²		
Lysis Block ¹	1 Plate	740 665.1		
MN Tube Strip Rack ¹	1 Rack	740 665.1		
200 µL Filtered Fine Bore Tips in Robotic Rack sterile	2 Racks	2097	Corbett Robotics	
Elution Plate with 0.65 mL Cluster Tubes ⁴	1 Plate	2147		
Elution Plate Strip Caps (1 bag contains 12 strips of 8) ⁴	1 Bag	1636		
Reagent Tubs	70 mL Tub	3 Tubs		2137 Disposable (2365 Reusable)
	170 mL Tub	2 Tubs		2136 Disposable (2364 Reusable)
	270 mL Tub	1 Tub		2314 Disposable (2363 Reusable)
Reagent Tub Lids	70 mL Tub Lid	2 Lids		2505 (for use with disposable tubs) (2416 Reusable)
	170 mL Tub Lid	3 Lids		2504 (for use with disposable tubs) (2415 Reusable)
	270 mL Tub Lid	1 Lid		2503 (for use with disposable tubs) (2414 Reusable)
Self adhesive PCR/Elisa plate Plastic Sealing Film (for sealing unused wells of capture plate)	1 Sheet	2411		

¹ Part of NucleoSpin® 96 Blood kit or NucleoSpin® 8 Blood kit, respectively.

² Kit is available for different number of preparations. See ordering information of kit protocol.

³ Required only when using 8-well strip format.

⁴ not required when using MN Tube Strip rack supplied with the NucleoSpin® 8/96 Blood kits.

Reagents and Consumables Required

Item	Requirement for a Full 96-Well or 48 Well Extraction	Part No. or Part of (MN)	Supplier
NucleoSpin® 96 Blood kit (including buffers, lysis block, MN Tube Strips for collection of eluted DNA) Sufficient for 1 x 96, 4 x 96 or 24 x 96 preps	1 kit	740 665.1 740 665.4 740 665.24	MN
NucleoSpin® 8 Blood kit (including buffers, MN Tube Strips for collection of eluted DNA) Sufficient for 12 x 8 preps or 60 x 8 preps NB: MN Accessory Starter set A required to use 8 Strip System (P/N 740 682)	1 kit	740 664 740 664.5	
<i>Additional reagents to be ordered separately if required</i>			
Buffer BQ1	125 mL	740 923	
Buffer BW	500 mL	740 922.500	
Buffer B5 (concentrate for 500 mL)	100 mL	740 921.100	

Accessories Required

Part No.	Corbett Robotics Description
1675	High Skirt Transfer Carriage
1697	96 well Separator Plate
1696	8 Strip Separator Plate NB: MN Accessory Starter set A required to use 8 Strip System (P/N 740682)
2443	Elution Riser Block (16.25 mm) Optional not required when using MN tube strip rack supplied with kit for Corbett Robotics elution tubes only
2139	Reagent Tub SBS Base plate

Reagent Handling and Storage

HAZARD INFORMATION

Buffers BQ1, BW contain guanidine hydrochloride, alcohols and detergents. Always wear a laboratory coat, disposable gloves, and eye protection when handling solutions containing these chemicals. Do not add bleach or acidic solutions directly to solutions containing guanidine or extraction waste. Guanidine forms reactive compounds and toxic gases when mixed with bleach or acids. For any items contaminated with these buffers, clean with suitable laboratory detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions. For details refer to the MSDS (material safety data sheet) information available at the following web site:

www.mn-net.com

Reagent storage

Upon receipt of reagents, unpack and store the individual reagents as follows:

	Store Temp	Storage State
Buffer BQ1	18–24°C	Dark
Buffer BW	18–24°C	Not critical Keep bottle tightly closed
Buffer B5	18–24°C	Not critical Keep bottle tightly closed
Buffer BE	18–24°C	Not critical
Buffer PB	18–24°C	Not critical
Proteinase K lyophilized or dissolved in buffer PB	4°C	Not critical

Reagent Preparation

Prior To Each Run

Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by warming to 37°C until dissolved. Swirl gently to avoid foaming.

Identifying Required Reagent Volumes for Your Run

The robotics software will calculate for you the exact required volume of each reagent once you have selected the number of columns you will be extracting from.

The X-tractor Gene™ software will display the required volume of each reagent (inclusive of each reagent tub's allocated dead volume) in a hover box when you place the computer's mouse cursor over the reagents designated position.

Proteinase K working solution

Dissolve Proteinase K with Buffer PB as indicated on label.

Lysis Reagent

For each sample mix 75 µL of Buffer BQ1 with 25 µL resuspended Proteinase K solution. Use lysis reagent immediately after preparation. (Prepare the required volume as indicated by the X-tractor Gene™ software @ 75% Buffer BQ1 plus 25% Proteinase K solution).

Binding Solution

For each sample mix 200 µL of Buffer BQ1 with 200 µL of 96-100% ethanol. (Prepare the required volume as indicated by the X-tractor Gene™ software @ 50% Buffer BQ1 plus 50% of 96-100% ethanol).

Wash Solutions

Buffer BW: ready to use.

Buffer B5: add 96-100% ethanol as indicated. Close bottle tightly in order to prevent ethanol evaporation.

Sample Collection and Storage

Sample collection

Collect blood into EDTA or Lithium Heparin Vacutainers. Invert gently 10 times then cool to 2–8°C. For Plasma serum or WBC/buffy coat, process the samples without delay.

Sample storage

Whole blood should be stored at 2–8°C and is viable for DNA extraction for up to 3 months, although some loss of DNA quality will occur over time. For long-term storage, freeze aliquots at -20°C or -80°C.

Plasma, serum or WBC/buffy Coat can be stored at 2–8°C for up to 6 hours. For long-term storage, freeze aliquots at -20°C or -80°C.

Frozen whole blood plasma or serum and other biological liquid samples must not be thawed more than once. Repeated freeze-thaw cycles leads to precipitates that will clog capture membranes and reduce yield.

See *Sample Preparation* below for details on how to remove precipitates.

Sample Preparation

General Considerations

Sample preparation on the X-tractor Gene™ should be conducted in the same manner as for spin columns. Difficulties in sample processing can be addressed in ways similar to spin columns. Common considerations are listed below. Please refer to the troubleshooting section if needed.

Empirically determine the maximum amount of lysate to load onto the column to ensure complete and consistent passage of your sample type.

IMPORTANT

Uniformity of samples is highly desirable

Blocked Wells

The capture plates' glass fibre membrane can be easily blocked by samples with particulate matter or high viscosity (highly concentrated DNA and DNA/protein lysates are usually very viscous).

DNA overloading of the membrane will also cause blockage, therefore apply no more than 1 × 10⁶ nucleated mammalian cells (approx 7 µg of DNA for most mammalian cells).

Once a membrane is blocked, buffer flow may halt and the well will require manual removal of most of the subsequent buffers loaded. Alternatively, the membrane can be pierced with a 27-gauge needle. Overloaded or blocked glass fibre membranes lead to reduced yields and low quality DNA.

Preparing Difficult Samples

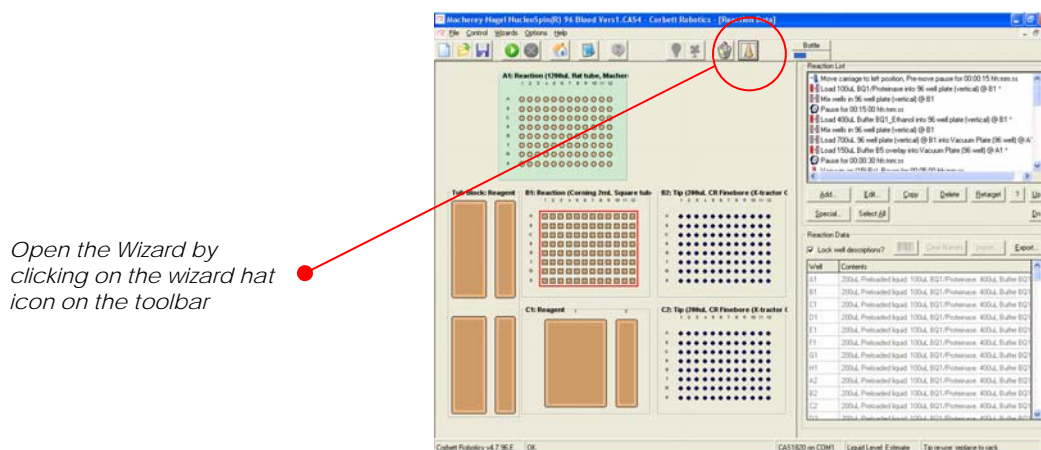
Avoid transferring material into the lysis block that could cause pipette blockage (e.g. blood clots).

To maximise nucleic acid recovery from difficult samples such as frozen blood or samples with precipitates, follow the suggested guidelines below:

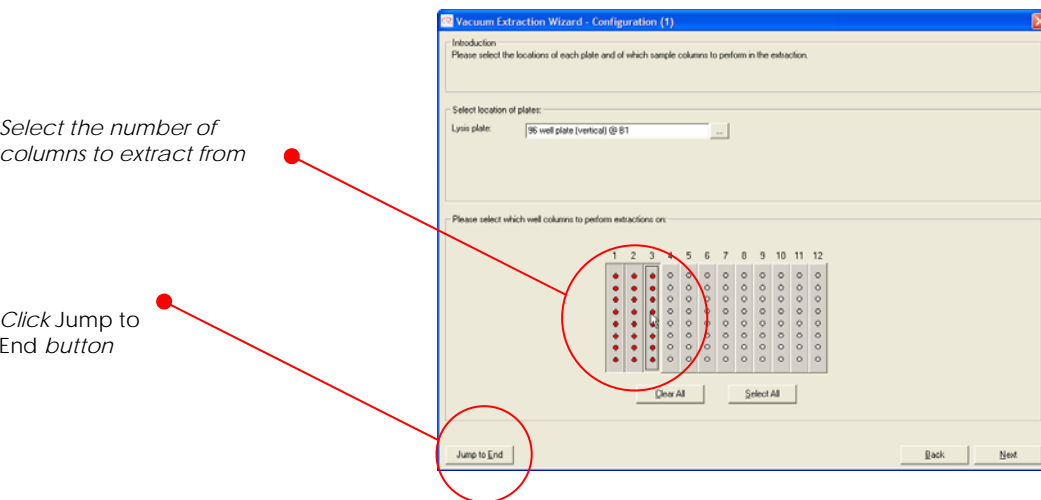
1. Seal and incubate the lysis block after addition of Buffer BQ1/Proteinase K for up to 1 hour at 56°C with constant agitation.
2. Centrifuge at 2,500 x g for 10 minutes to pellet remaining clots and precipitates.
3. Aspirate 300 µL of the digest supernatant off the pellet and mix with 400 µL of Lysis Buffer as described in the second step of the extraction protocol.

Setting Up to Execute a Run

1. Turn on X-tractor Gene™.
2. Launch software.
3. Open the *Macherey-Nagel NucleoSpin(R) 96 Blood V01.CAS4* run file (for 96-well plates).
Open the *Macherey-Nagel NucleoSpin(R) 8 Blood V01.CAS4* run file (for 8-well strip format plates).
4. Select the number of columns to extract from:



Open the Wizard by clicking on the wizard hat icon on the toolbar



Select the number of columns to extract from

Click Jump to End button

5. Once you have selected the required columns, exit the Wizard by clicking on the *Jump to End* button in the bottom left hand corner of the *Configuration* window.
6. This will take you to the *Review Protocol* screen. Confirm the protocol. If required, this screen can be printed to assist in robot setup or for your records. Click *Next*, and then *OK* to exit the Wizard.

The X-tractor Gene™ will now calculate the required volumes of the described reagent for each tub and the number of tips required.

Loading Workspace Prior to a Run

Load Samples into Lysis Block

1. Add 200 µL sample (see *Sample Preparation* for advice) to each lysis block well for the columns you wish to extract from.
2. Load the lysis block onto the B1 position of the X-tractor Gene™.
3. If you have less than 96 samples to extract you will need to cover the unused portion of the capture plate with a piece of self adhesive sealing film. Covering the unused portion is essential for proper vacuum operation. DO NOT attempt to re-use the unused portion of the plate as repeated handling of the capture plate can result in cross-contamination of subsequent extractions.
4. If the sample volume is less than 200 µL bring the volume up to 200 µL with PBS buffer.
5. Re-suspend any cell pellets to 200 µL as above.
6. Ensure the separator plate is thoroughly clean and dry (see *Cleanup*).
7. Observing sterile procedure set up the instrument deck with clean accessories, the required consumables and all reagents with the exception of the Lysis and binding solutions.
8. Fill the reagent tubs with the indicated volumes of in the following order, and cover the tubs with the supplied reagent tub lids.
9. Prepare these buffers first:
 - a. Buffer B5 Overlay
 - b. Buffer B5
 - c. Buffer BW
 - d. Elution Buffer BE

WARNING

Do not dispense the required volumes of reagents into the reagent tubs until just prior to the start of the run.

Keep reagents covered with the provided lids until you are ready to start the run. Leaving reagents in tubs for extended periods will result in evaporation (especially of alcohol solutions) and salt precipitate formation resulting in loss of binding conditions. For this reason reagents left over from a previous run should be disposed of and new or clean tubs loaded onto the deck with fresh reagents.

10. Then
 - a. Prepare the required volume of Binding Solution: Dilute the Lysis Buffer BQ1 with Ethanol at a ratio of vol/vol 50% Lysis Buffer BQ1 to 50% Ethanol and load into designated reagent tub (cover the tub with the supplied reagent tub lid).

WARNING

Do not add the Proteinase K solution to Lysis solution until just before you start the run and you have completed and checked all the run preparations.

- b. Prepare the required volume of Lysis solution: (75% Lysis Buffer BQ1 to 25% Proteinase K solution vol/vol), pipette the Proteinase K into a 15 mL falcon tube then add the required volume of Lysis Buffer BQ1, mix gently by inverting the tube 10 times, taking care to avoid foaming. Immediately pour buffer into the assigned reagent tub and cover the tub with the supplied reagent tub lid.

11. Start the run.

Run Protocol

Pre-Run Checklist

Click the *Start* icon and a pre-run checklist will appear. The checklist may include some of the following instructions.

Please ensure:

- That the columns you wish to extract from are correctly selected and the unused portion of capture plate is sealed with self-adhesive microtiter plate plastic sealing film or PCR foil.
- That the elution plate is in position and its lid removed.
- Sufficient pipette tips are loaded and lids removed.
- Clean reagent tubs are loaded with the required volume of reagent, are in the correct positions, and their covers are on.
- All reagents and samples are at room temperature at the start of the run.

For first time users, the software option of user pause at the end of vacuum steps is enabled; this requires the user to confirm that all the samples have flowed through the capture plate before continuing. This option can be turned off once proper sample preparation has been confirmed.

Start Run

Once you have confirmed that the X-tractor Gene™ workspace is correctly set up as described in the software and confirmed the pre-run checklist, click *Run* and the protocol will execute as described below.

Load Lysis Solution

- Add 100 µL of Lysis Solution (75 µL Buffer BQ1 + 25 µL Proteinase K) to 200 µL of sample.
- Mix 5 times.
- Incubate at room temperature for 15 minutes.

To maximise DNA recovery from difficult samples (i.e. frozen blood, or samples with clogging precipitates), incubate sealed plate for 1 hour @ 56°C with constant agitation. Centrifuge @ 2,500 x g for 10 minutes to pellet remaining clots and precipitates, aspirate the supernatant off the pellet and mix 300 µL of digest supernatant with Binding Solution as described below. You may need to increase the digest volumes to 150 µL of Lysis solution and 300 µL of Sample.

Load Binding Solution

- Add 400 µL of Lysis Buffer (Buffer BQ1/Ethanol Mix).
- Mix 5 times.
- Incubate at room temperature for 5 minutes.

Load Filter Plate plus B5 Overlay (Wash 1)

- Load 700 µL of lysate into the capture plate.
- Pre-mix 2 times.
- Overlay with 150µL Buffer B5 (no vacuum after loading sample allows you using the wash 1 screen to overlay the sample with Buffer B5).
- Apply a vacuum of 15 kPa for 5 minutes (check for slow or blocked wells).

Wash Step 2

- Load 600 µL of Wash Buffer BW into the capture plate.
- Apply a vacuum of 20 kPa for 3 minutes.

Wash Step 3

- Load 900 µL of Wash Buffer B5 into the capture plate.
- Apply a vacuum of 20 kPa for 1 minute.
- Repeat 1 time (2 iterations total).

Dry Sample

- Apply a vacuum of 60 kPa for 10 minutes.

Product Removal

- Load 100 µL of BE Buffer (Elution Buffer) into the capture plate.
- Incubate for 5 minutes.
- Apply a vacuum of 60 kPa for 1 minute.
- Repeat 1 time (2 iterations total).

Finish

- Recover elution plate with samples from the elution chamber.

- Remove and discard used consumables and clean separator plate, sink, tubs and carriage in preparation for the next run.
- (Please refer to Appendix A for nucleic acid storage advice).

Post-Run Cleanup

Disposable Plasticware and Liquid Waste

Dispose of plasticware and liquid waste in accordance with laboratory guidelines for the sample type and reagent hazard.

WARNING

Do not add bleach or acidic solutions directly to solutions containing guanidine or extraction waste. Guanidine forms reactive compounds and toxic gases when mixed with bleach or acids. For any items contaminated with these buffers, clean with general laboratory detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.

Transfer carriages, waste sink and tip chute

Thoroughly rinse under cold tap water and allow to dry. Do not apply hot water to these components.

Separator Plates and Non-disposable Reagent Tubes

The Separator Plate and non disposable reagent tubes must be washed to ensure they are RNA/DNA and RNase/DNase-free. Ensure they are dry before re-using.

Do not autoclave or heat sterilize separator plate and non disposable reagent tubes (do not exceed 100°C)

When washing the separator plate, scrub lightly with a brush, this will help dislodge air bubbles that can become trapped in the holes and prevent the plate from being cleaned thoroughly. Agitating the plate up and down will also help ensure the holes are properly washed.

1. First rinse with tap water to remove any guanidine salts.
2. Then to clean either soak in 1 % sodium hypochlorite (final concentration of bleach) for >30 minutes, then rinse thoroughly with large amounts of milliQ or Molecular Biology grade RNase-free water.

Or

Soak for 1 minute in 0.1 M NaOH, 1 mM EDTA followed by a 1 minute soak in 0.4 M HCl then rinse thoroughly with large amounts of milliQ or Molecular Biology grade RNase-free water.

Alternatively, the plate may be cleaned with DNA Zap (Ambion Inc, Austin, TX; P/N 9890).

Troubleshooting

Problem	Cause	Solution
Slow or blocked capture plate wells	Excessive sample	Reduce the amount of starting material. Typically, 1×10^6 nucleated cells is optimal. To preserve the sample you are currently working with, remove it from the well and re-run on a different capture plate. Once you have recovered the sample, pierce the bottom of the glass fibre membrane through the nozzle with a 27-gauge needle to allow subsequently loaded buffers to pass through.
	Incomplete lysis of sample material	Increase incubation time and temperature with the Lysis Solution. Increase mixing. See: incomplete digestion or lysis of samples (below).
	Particulates, precipitates and clots blocking membrane	Avoid clots when aspirating sample into lysis block. Pellet particulates (2,500 x g, 10 minutes) and load sample supernatant into lysis block. Digest precipitates then pellet undigested material (2,500x g, 10 minutes), load digested sample supernatant into a fresh lysis block.
	Vacuum too low	Increase the vacuum or prolong the vacuum time. Ensure the vacuum applied complies with the extraction protocol.
Incomplete digestion or lysis of samples	Proteinase K was not added to the digestion	Add the Proteinase K solution to the buffer BQ1 to prepare Lysis Solution just prior to the run.
	Proteinase K activity too low	Add Proteinase K solution to Buffer BQ1 immediately prior to run (Proteinase K should not be in Buffer BQ1 for not more than 10 minutes before being diluted with sample). Freeze-thaw cycles of Proteinase K solution reduce the activity of the Proteinase K; repeat with a freshly prepared proteinase K solution. Completely mix the Proteinase K solution with the Buffer BQ1 by inverting tube 10 times.

Troubleshooting

Problem	Cause	Solution
Poor DNA yield	Incorrect type or quality reagent or plasticware	Before continuing, it is essential you use original and fresh MACHEREY-NAGEL reagents and the recommended Corbett consumables (see section <i>Reagents and Consumables Required</i>).
	Incomplete digestion or lysis of samples	See: Incomplete digestion or lysis of samples (above).
	Particulates, precipitates and clots blocking membrane	See: Particulates, precipitates and clots blocking membrane (above).
	Incomplete elution	<p>Prolong the incubation time with Buffer BE (Elution Buffer), or repeat the elution step again. Incubate the Buffer BE (Elution Buffer) at 70°C.</p> <p>Check well was not overloaded.</p> <p>Confirm the DNA was eluted in 200 µL (2 x 100 µL).</p> <p>Increase elution vacuum time in preference to increasing vacuum pressure.</p>
	Sample is old or degraded	Collect process and store samples correctly as soon as possible after collection.
	Sample has a low nucleated cell count	<p>Prepare buffy coat.</p> <p>Increase sample size if possible and other constraints allow (i.e. flow rates and well volumes).</p>
	Water was used for elution instead of elution buffer	<p>Buffer BE (Elution Buffer) is recommended for optimal yields and storage of the purified DNA. If water is used to elute DNA, confirm that the pH is at least 7.0, as elution efficiency falls dramatically below pH 7.0 and acidic conditions may subject the DNA to acid hydrolysis when stored for long periods of time.</p> <p>Note: stored water can become acidic over time.</p>
	Using lysate stored at -20°C or -70°C	Lysate that has been frozen may have a decreased amount of genomic DNA. For optimal performance, purify the DNA as soon as the lysate is prepared
Samples have undergone multiple freeze-thaw cycles	Samples that have been frozen and thawed repeatedly will eventually experience DNA degradation. Use fresh samples where possible.	

Troubleshooting

Problem	Cause	Solution
Poor DNA yield	Sample size low in genomic DNA	The yield of genomic DNA may vary depending on the number of cells used for the isolation and size of the genome of organism. Aim for a maximum sample size of 1×10^6 nucleated mammalian cells or, for other organisms, sufficient cells to give no more than 7 μg of DNA.
	Excessive evaporation of reagents	The required volumes of reagents should not be dispensed into the reagent tubs until just prior to the start of the run. Leaving reagents in open tubs for extended periods will result in evaporation of water and alcohols. This will result in salt precipitates and loss of poor binding conditions
	Steps not followed correctly or wrong reagents used	This protocol requires that the correct volumes of reagents are used in a specific order. When done correctly the DNA will bind and remain bound to the membrane during the purification.
Low DNA purity (A260/280 ratio too high)	Genomic DNA is contaminated with RNA	Include an RNase step in future isolations.
Inhibition with downstream applications	Incorrect type or quality reagent or plasticware	Before continuing, it is essential to ensure you use original and fresh MACHEREY-NAGEL reagents and the recommended Corbett consumables (see section <i>Reagents and Consumables Required</i>). Low quality chemicals may cause inhibition effects, as can inhibitors leached from incorrect plasticware.
	Salt carryover during elution	Check Wash Buffer for salt precipitates. If there are any precipitates, carefully warm until they dissolve.
	Ethanol carry over during elution	Increase drying time for ethanol removal step. Dry plate at 50°C for 10 minutes.
	Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the amount of your eluate added accordingly.

	<p>Elution cluster rack tubes autoclaved before elution</p>	<p>Do not autoclave elution cluster rack tubes. This may leach chemicals from the tubes, which may inhibit enzymatic reactions.</p> <p>Repeat the purification with a new set of elution cluster rack tubes.</p> <p>The Corbett Robotics or MACHEREY-NAGEL cluster rack tubes are RNase/DNase and RNA/DNA free.</p>
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Nucleic Acid Storage

A working stock of DNA can be stored at 2 – 4 °C for several weeks. For long term storage DNA should be stored at -20 °C.

RNA should be stored at -80 °C at all times (and held at 2 – 4 °C during use).

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Please note that the elution buffer BE supplied within the NuceloSpin® 8/96 Blood kits contain 5 mM Tris pH 8.5.

Repeated freeze thaw cycles should be avoided as this can share the DNA.

Disclaimers

Protocol Use

It is the user's responsibility to validate performance of this protocol for any particular application, since performance characteristics of this protocol and its product have not been validated for any specific application.

This protocol is for *in vitro* research use only.

It is not intended to identify any specific organism or for clinical use.

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