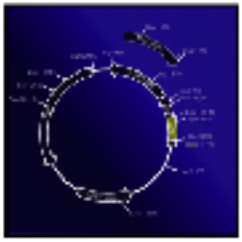
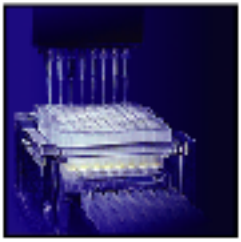




Plasmid and large-construct DNA Purification

User manual

NucleoSpin® 96 Flash

February 2006/Rev. 02

MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoSpin® 96 Flash		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**[1]
Cat. No.	**740618.2**	**740618.4**	**740618.24**
Buffer F1	125 ml	250 ml	6 x 250 ml
Buffer F2	125 ml	250 ml	6 x 250 ml
Buffer F3	125 ml	250 ml	6 x 250 ml
Buffer FE	60 ml	60 ml	6 x 60 ml
RNase A (lyophilized)[2]	25 mg	50 mg	6 x 50 mg
MN Square-well Block (culture plate)	2	4	24
Square-well Block (precipitation plate)	2	4	24
Gas-permeable Foil[3]	2	4	24
Self-adhering Foil[4]	10	20	120
NucleoSpin® Flash Filter Plates	2	4	24
Protocol	1	1	6 x 1
Register	1	1	6

[1] The kit for 24 x 96 preparations (Cat. No. 740 618.24) consists of 6 x Cat. No. 740 618.4
[2] For preparation of working solutions and storage conditions see section 3.
[3] Every MN Square-well Block (culture plate) includes a Gas-permeable Foil.
[4] For ordering information please contact Technical Service. Use of Self-adhering PE Foil (Cat. No. 740676) is not recommended.

2 Product description

2.1 The basic principle

With the **NucleoSpin® 96 Flash** procedure, plasmid DNA is liberated from the *E. coli* host cells by SDS/alkaline lysis after resuspension of the pelleted bacteria (buffers F2 and F1, respectively). The resulting lysate is then neutralized by addition of F3 – cell debris will precipitate together with SDS. Incubation in boiling water (optional) inactivates nucleases which are present in a variety of *E. coli* host strains and denatures soluble proteins and other cell constituents. All precipitates are then removed by filtration of the lysate through the **NucleoSpin® 96 Flash** Filter Plate under vacuum or in a suitable centrifuge for microtiter plates. Plasmid DNA is then precipitated by addition of isopropanol to the filtrate and subsequent centrifugation. After an additional washing step with 70% ethanol and drying, the DNA can be resuspended in buffer FE (5 mM Tris-Cl, pH 8.5).

2.2 Kit specifications

- **NucleoSpin® 96 Flash** is designed for the rapid manual and automated 96-well DNA preparation of high and low copy-number plasmids and large low-copy constructs (e.g. BACs, bacterial artificial chromosomes) DNA from *E. coli* cultures.

- For a support protocol for isolation of DNA from BACs see section 6.3.

- The kit is for use under vacuum (common laboratory automation workstations; see section 2.5), Nucleo-Vac 96 vacuum manifold (see ordering information) or similar suitable vacuum manifolds (see section 2.3) or in a centrifuge (see section 2.4). The final precipitation requires the use of a centrifuge capable to spin a square-well block with at least 2,500 x *g*.

- The kit allows the rapid parallel purification of up to 8 µg of highly pure plasmid DNA from 1.1 – 1.3 ml of a saturated *E. coli* culture per preparation in the convenient 96-well format.

- The prepared plasmid DNA is suitable for many automated fluorescent DNA sequencing applications, PCR, or many types of enzymatic manipulation.

- Time for manual parallel processing of up to 384 plasmid DNA minipreps from *E. coli* cultures with **NucleoSpin® 96 Flash** is less than 90 min.

Table 1: Kit specifications at a glance	
	NucleoSpin® 96 Flash
Culture volume	1.1-1.3 ml
Average yield	8 µg (plasmids)
	0.2 µg (BACs)
Vectors	up to 250 kb
Time/prep	90 min/4 x 96

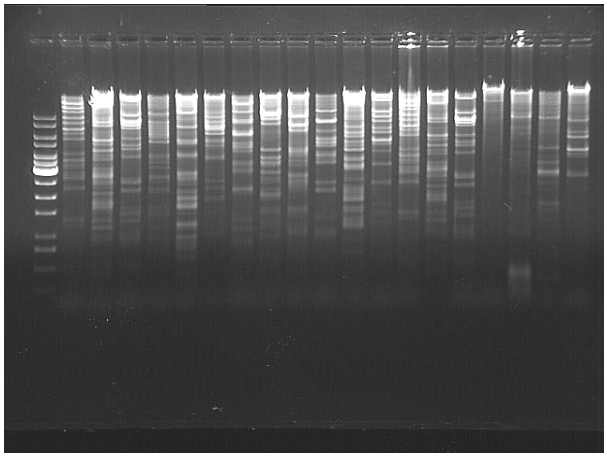


Fig 1: Isolation of BAC DNA (pBeloBacKan derivatives) and restriction analysis.
BAC DNA was isolated acording to the support protocol for BAC DNA purification. DNA was resuspended in 20 µl resuspension solution FE. 10 µl were digested with 5 U *Eco*R1 for 1 h at 37°C. Samples were separated on 0.7 % agarose gel (ethidium bromide stain).
Lane 1: 1 kb ladder MBI fermentas.

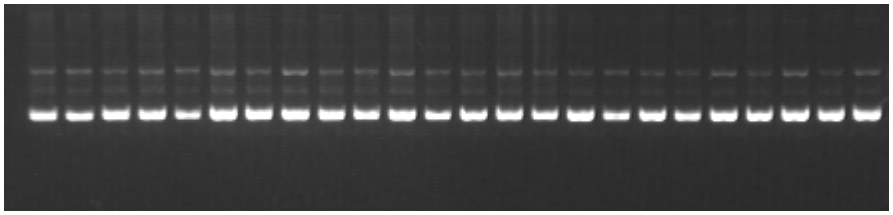


Fig 2: Purification of high copy number plasmids pBluescript SK-
DNA was isolated according to the standard protocol. Finally, DNA was resupended in 75 µl buffer FE. The figure shows 24 represantive samples derived from the **NucleoSpin® 96 Flash** kit. 10 µl were analyzed by separation on a 1 % agarose gel and stained with ethidium bromide.

2.3 Suitability for other common vacuum manifolds

The **NucleoSpin® 96 Flash** kit can be used with other common vacuum manifolds. For further details see list below.

Vacuum manifold	Suitability	Additional equipment
Qiagen/QIAvac 96	yes	no
BioRad/Aurum vacuum manifold	no	
Eppendorf/Perfect VAC Manifold	no	
Millipore/MultiScreen	no	

2.4 Required additional equipment

Harvesting of bacterial cells and plasmid precipitation is achieved in a centrifuge with a swinging-bucket rotor attaining ≥ 2,500 × *g*. Clearance of the buckets must be sufficient to accommodate square-well blocks (height: 44 mm). For clearing of the neutralized and heat-incubated lysate, a NucleoVac 96 vacuum manifold is required for manual use of the **NucleoSpin® 96 Flash** kit.

If using a centrifuge with a swing-out rotor capable to accommodate the NucleoSpin® Flash Filter Plate/square-well block sandwich (bucket height: 85 mm), e.g. Hermle Z 513/Z 513 K, Jouan KR4i, Heraeus Kendro Multifuge 3/3-R, Beckman Coulter Allegra 25R, Hettich Rotanta 460 series, Sigma 4-15/4K15/6-15/6K15 no NucleoVac 96 vacuum manifold is necessary and the whole procedure can be performed in the centrifuge. Shorter centrifugation times are then sufficient, if this centrifuge is capable of attaining 6,000 × *g*.

2.5 Automation

NucleoSpin® 96 Flash is designed for use on common laboratory automation workstations, such as:

Robot Supplier	Robot
Beckman-Coulter	Biomek 2000/FX
Cavro	MiniPrep series
Hamilton	Microlab Star

Robot Supplier	**Robot**
MWG	RoboSmart/RoboPrep
Perkin Elmer	MultiPROBE II/II HT
Qiagen	BioRobot 9600/3000/8000
Tecan	Genesis RSP/RWS Separation System series
Zymark	SciClone ALH

Note: As other laboratory automation workstations are currently under evaluation please contact MN directly if your workstation is not on this list.

Visit MN on the internet at *www.mn-net.com* or contact your local MACHEREY-NAGEL distributor for availability of ready-to-run scripts and for technical support regarding hardware, software, setup instructions, and selection of the protocol. All MN protocols can be downloaded from our website.

3 Storage conditions and preparation of working solutions

Attention:

Buffer F2 contains SDS and sodium hydroxide which are irritant and hazardous. Wear gloves and goggles when handling them.

- Store bottles tightly closed at all times. Buffer F2 will absorb CO_2 if exposed to air. This leads to a decreasing pH, resulting in suboptimal kit performance.

- Sodium dodecyl sulfate (SDS) in buffer F2 may precipitate if stored at temperatures below 20°C. If a precipitate is observed in buffer F2, incubate the bottle at 30 – 40°C for some minutes and mix well until all of the precipitation is redissolved.

- If you are using the **NucleoSpin® 96 Flash** manually establish a reliable vacuum source for the NucleoVac 96 manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of 200-400 mbar (pressure difference). The use of the NucleoVac Vacuum Regulator (see ordering information) is recommended. Alternatively adjust vacuum that during the purification the sample flows through the column with a rate of 1-2 drops per second

Before starting any **NucleoSpin® 96 Flash** protocol prepare the following:

- Before first use of the kit, add RNase A to buffer F1: Add 1 ml F1 to the RNase A vial, vortex, and pipette all of the resulting solution to the F1 bottle. The RNase in the mixture is stable for six months if stored at +4°C.

4 Safety instructions - risk and safety phrases

The following components of the NucleoSpin® 96 Flash kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
RNase A	RNase A, lyophilized	Xi*	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24
F2	sodium hydroxide < 2 %	Xi**	Irritating to eyes and skin	R 36/38	S 26-37/39-45

Risk Phrases

R 36/38 Irritating to eyes and skin

R 42/43 May cause sensitization by inhalation and skin contact

Safety Phrases

S 22 Do not breathe dust

S 24 Avoid contact with the skin

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S 37/39 Wear suitable gloves and eye/face protection

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

* Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

** Label not necessary, if quantity below 25 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 Growing of bacteria cultures

5.1 Selection of culture medium

The cultivation of cells is recommended at 37°C in LB (Luria-Bertani) medium at constant shaking (200-250 rpm). Alternatively, rich media like 2 x YT or TB (Terrific Broth) can be used. By using 2 x YT or TB, bacteria grow faster and reach the stationary phase much earlier than in LB medium (≤ 12 h). This may lead to a higher percentage of dead or starving cells when starting the preparation. The resulting plasmid DNA from overgrown cultures may be partially degraded or contaminated with chromosomal DNA.

5.2 Cultivation of bacteria in a MN Square-well Block

Autoclave the MN Square-well Block supplied with the kit or use a sterile block. Add 1.2-1.5 ml of selected medium (with appropriate antibiotic, e.g. 100 µg/ml ampicillin) to each well of the MN Square-well Block. To avoid cross-contamination due to spillage during incubation, do not exceed a total culture volume of 1.5 ml. Inoculate each well with a single bacterial colony. Cover the MN Square-well Block with the Gas-permeable Foil supplied with the MN Square-well Block. Grow the culture in a suitable incubator at 37°C for 16-24 h with vigorous shaking (200-400 rpm). The MN Square-well Block may be fixed to the shaker with large-size flask clamps (for 2-l flasks) or tape.

Note:
The yield of plasmid DNA depends on growth conditions, bacterial strain, and cell density of the culture as well as on the size and copy number of the vector. Use of high copy-number plasmids such as pUC, pBluescript or pGEM, and E. coli strains like DH5α or XL1 Blue are recommended. Growth times of 16-24 h are usually sufficient. However, for poorly growing bacteria, prolonged incubation times of up to 30 h may be required.

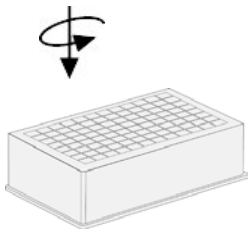
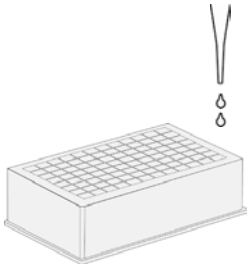
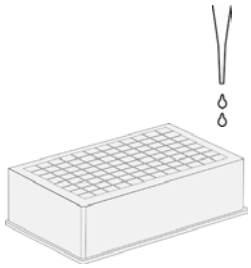
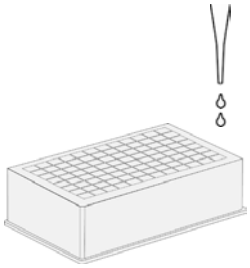
5.3 Cultivation of bacteria in tubes

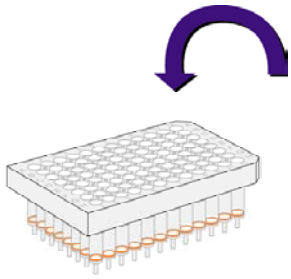
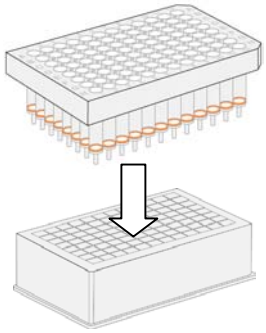
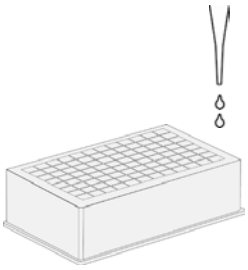
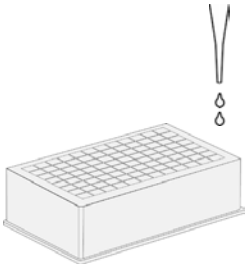
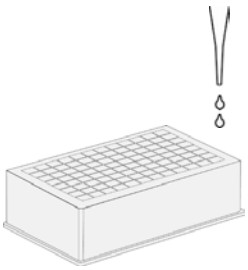
Use 1-5 ml of appropriate culture medium. Depending on the bacterial strain and copy number of the plasmid up to 5 ml LB medium or 3 ml 2 x YT or 3 ml TB medium can be used. Grow bacteria with vigorous shaking for 10-14 h.

Optional:
Transfer bacterial culture from the tubes to the MN Square-well Block supplied with the kit. For this, transfer 1.5 ml of the culture to each well of the MN Square-well Block. Harvest the cultures by centrifugation. Discard supernatant. Usually 1.5 ml of culture is sufficient for DNA preparation. However, if necessary, add an additional 1.0-1.5 ml of the bacteria culture to each well of the MN Square-well Block, centrifuge again, and discard the supernatant.

Do not use more than 5 ml LB culture or 3 ml rapid growing bacterial strain (using 2 x YT or TB medium) because lysis efficiency might be lower when using cell pellets which are too large.

6 General procedure

1	**Cultivate** and **harvest** bacterial cells	**LB** **2 x YT** **TB** **10 min** **1,000 x *g***	
2	**Resuspend** bacterial cells	**300 µl F1** **mix or shake**	
3	**Lyse** bacterial cells	**300 µl F2** **RT** **2-5 min** **(optional: shake)**	
4	**Neutralize**	**300 µl F3** **(optional: mix or shake)**	
5	**Heat incubation (optional)**	**100°C** **5 min**	

6	**Transfer** of crude lysates to NucleoSpin® Flash Filter Plate (orange)		
7	**Clear** crude lysates directly into Square-well Block	**ca. - 0.2 to - 0.4 bar*** **(1 min to 5 min)** **or** **1,500 x *g*** **(1 min to 5 min)**	
8	**Precipitate DNA**	**630 µl isopropanol (0.7 volumes)** **15 min** **≥ 2,500 x *g***	
9	**Wash pellet**	**700 µl ethanol (70%)** **3 min** **≥ 2,500 x *g***	
10	**Dry pellet**	**55°C** **10 min**	
11	**Resuspend DNA**	**20-50 µl FE**	

* reduction of atmospheric pressure

6.1 Standard protocol for manual purification of high-copy plasmid DNA

1 Cultivate and harvest bacterial cells

Pellet the bacteria by centrifuging the MN Square-well Block (leave Gas-permeable Foil on the block) at **1,000** × ***g*** for **10 min**. Remove the Gas-permeable Foil and invert the block quickly to pour off the supernatant. Tap the block on a paper sheet or paper towels in order to remove as much of the remaining medium as possible.

2 Resuspend bacterial cells

Add **300 µl F1** to each bacterial pellet and resuspend the cells by vortexing.

Be sure that all of the RNase A has been added to buffer F1 (see section 3). Vortex the block until no more cell clumps or pellets are visible in the wells.

3 Lyse bacterial cells

Add **300 µl F2** to each well. Seal the block thoroughly with Self-adhering Foil and make sure the foil sticks to the walls between the wells to prevent well-to-well cross contamination. Mix by gently inverting the block **3 – 4 times**. Incubate at room temperature for up to **5 min.** ***Do not vortex.*** Remove Self-adhering Foil and dry the upper rim of the wells with a paper towel.

The bacterial suspension should become clear as cell lysis occurs. Vortexing at this step will shear the genomic bacterial DNA, leading to contamination of the resulting plasmid DNA preparation. Do not extend the cell lysis to more than 5 min.

4 Neutralize

Add **300 µl F3** to each well. Seal the block thoroughly with Self-adhering Foil and make sure the foil sticks to the walls between the wells to prevent well-to-well cross contamination. Mix gently by inverting **3 – 4 times**.

A white precipitate of SDS and cell debris forms.

5 Heat incubation (optional)

If using endA$^+$ host strains: Seal the block with a fresh Self-adhering Foil and incubate it in a boiling water bath for **5 min**. Let the block cool down on ice to at least room temperature.

This heating step can be omitted when using endA$^-$ host strains like DH5α or XL-1 Blue. Check with the host/plasmid system you use if boiling is necessary. When using endA$^+$ strains like E. coli *HB101, heat destruction of endonuclease activities is essential. Another benefit of heat treatment is denaturation of proteins and other cell constituents that have not been precipitated by alkaline lysis, making their removal during the following filtration step possible.*

Prepare the NucleoVac 96 vacuum manifold:

Insert spacers for “Square-well blocks”, notched side up, into the grooves located on the short sides of the manifold. Place a new Square-well Block into the manifold base, close the lid and insert the NucleoSpin® Flash Filter Plate into the lid.

*If using a centrifuge with a swing-bucket rotor capable of hosting 96-well blocks plus the NucleoSpin® Flash Filter Plate (e.g. NucleoSwing Z513), place the NucleoSpin® Flash Filter Plate on a fresh Square-well Block, apply lysates from step 4, and centrifuge for **2 min** at **2,500 × g.** Proceed directly to step 8.*

6 Transfer of crude lysates

Load the cell lysates into the wells of the NucleoSpin® Flash Filter Plate.

7 Clear crude lysates

Apply moderate vacuum (**0.2 – 0.4 bar** reduction of atmospheric pressure) until all of the samples have passed through the column (typically **< 5 min**).

It may be necessary to cover unused wells with a rubber pad if the vacuum does not build up immediately.

*If using a centrifuge with a swing-bucket rotor capable of hosting 96-well blocks plus the NucleoSpin® Flash Filter Plate (e.g. NucleoSwing Z513), place the NucleoSpin® Flash Filter Plate on a fresh Square-well Block, apply lysates from step 4, and centrifuge for **2 min** at **2,500 × g.***

8 Precipitate DNA

Remove the Square-well Block from the vacuum manifold. Add **630 µl** (0,7 volumes) of **isopropanol** to each well. Close the block with a Self-adhering Foil and mix by inverting the block **6 – 8** times.

Centrifuge the block for **15 min** at ≥ **2,500** × ***g***.

If using a NucleoSwing Z513 centrifuge or other suitable centrifuges (see section 2.5), spin the block for 10 min at 6,000 × g.

9 Wash pellet

Remove foil and discard the supernatant by inverting the block quickly. Tap the block on blotting paper or paper towels.

Add **500 µl ethanol** (**70%)** per well and seal block again. Centrifuge at **2,500** × ***g*** for **3 min**.

10 Dry pellet

Remove foil and discard the supernatant as described in step 9. Tap the block on paper sheet or paper towels. Dry the pellets at **50 – 55°C** for **10 – 15 min** in an incubator or oven until no more ethanol droplets are visible.

Ethanol is an inhibitor of many enzymatic reactions. Drying of the pellets is also possible at room temperature. However, for optimal removal of ethanol from the preparation, heat incubation is recommended.

11 Resuspend DNA

Add **50 µl FE** resuspension buffer (5 mM Tris-HCl, pH 8.5) and vortex the block briefly.

Optional:

*Close the block again with a foil and incubate at **50 – 55°C** for **10 - 15 min**. Centrifuge briefly before removing the foil. This step is recommended to speed up the resuspension of DNA if you intend to use it for immediately following downstream applications.*

If higher DNA concentrations are desired, the plasmid DNA can be resuspended in 20 µl FE as well. The sealed block can conveniently be used for storage of the plasmid DNA at +4°C or –20°C.

Quantify the yield of plasmid DNA by agarose gel electrophoresis before setting up sequencing reactions: compare aliquots of the minipreps to supercoiled plasmid DNA standards with known concentration. We recommend usage of ~1 µg of plasmid DNA for fluorescent dye primer and ~0.5 µg for fluorescent dye terminator cycle sequencing.

6.2 Standard protocol for automated purification of high-copy plasmid DNA

Note:
The list numbers in this protocol do not correspond with the list numbers in section 6 “General procedure”.

1 Cultivate and harvest bacterial cells

Pellet the bacteria by centrifuging the MN Square-well Block (leave gas permeable foil on the block) at **1,000 × *g*** for **10 min**. Remove the cover foil and invert the block quickly to pour off the supernatant. Tap the block on blotting paper or paper towels in order to remove as much of the remaining medium as possible.

It is strictly recommended to centrifuge the bacteria cultures under these conditions. Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Optional:

If centrifugation at higher g-forces is used, a shaker integrated on the robot worktable will be necessary for complete resuspension of the bacterial pellet after addition of buffer F1.

2 Before placing the MN Square-well Block (culture plate) in the desired position of the robot worktable, briefly vortex the block on a suitable vortexer.

This brief vortexing step will ensure an easy and complete resuspension of the pellet after addition of buffer F1.

3 Prepare buffer F1 by adding RNase A (see section 3).

4 Add buffers to the reservoirs or place the buffer bottles in the corresponding positions of the robot worktable. Place the plastic equipment like plates and the assembled vacuum manifold in the locations as specified in the individual robot program.

Optional:

The resuspension buffer FE (5 mM Tris/HCl, pH 8.5) may be substituted by nuclease-free water (check pH is 8.0–8.5 before use). This is recommended if the eluted DNA has to be concentrated for downstream applications or Tris salts interfere with downstream applications. A concentration of Tris higher than 10 mM can interfere with common sequencing chemistries.

5 Select method or program for DNA purification.

Optional:

After transfer to the NucleoSpin® Flash Filter Plate, incubate crude lysates for 1-3 min on the plate. This incubation allows the formation of a compact white precipitate. This step is usually not required for culture volumes up to 1.5 ml.

6 After addition of 0.7 volumes of Isopropanol to each well by the robot remove the Square-well Block from the vacuum manifold.

7 Centrifuge the block for **15 min** at ≥ **2,500** × ***g***. Remove foil and discard the supernatant by inverting the block quickly. Tap the block on blotting paper or paper towels.

*If using a NucleoSwing Z513 centrifuge, spin the block for **10 min** at **6,000** × **g.***

8 Add **500 µl ethanol** (**70%)** per well and seal block again. Centrifuge at **2,500** × ***g*** for **3 min**. Remove foil and discard the supernatant as described above (step 7). Tap the block on blotting paper or paper towels. Dry the pellets at **50 - 55°C** for **10 – 15 min** in an incubator or oven until no more ethanol droplets are visible.

Ethanol is an inhibitor of many enzymatic reactions. Drying of the pellets is also possible at room temperature. However, for optimal removal of ethanol from the preparation, heat incubation is recommended.

9 Add **50 µl FE** resuspension buffer (5 mM Tris-HCl, pH 8.5) and vortex the block briefly.

Optional: Close the block again with a foil and incubate at **50 – 55°C** for **10 - 15 min**. Centrifuge briefly before removing the foil. This step is recommended to speed up the resuspension of DNA if you intend to use it for immediately following downstream applications.

If higher DNA concentrations are desired, the plasmid DNA can be resuspended in 20 µl FE as well. The sealed block can conveniently be used for storage of the plasmid DNA at +4°C or –20°C.

10 Quantify the yield of plasmid DNA by agarose gel electrophoresis before setting up sequencing reactions: compare aliquots of the minipreps to supercoiled plasmid DNA standards with known concentration. We recommend usage of ~1 µg of plasmid DNA for fluorescent dye primer and ~0.5 µg for fluorescent dye terminator cycle sequencing.

6.3 Support protocol for automated and manual purification of large low-copy construct DNA

Recommendations for cultivation of large low-copy constructs, e.g. BACs:

- Use a freshly prepared preculture to inoculate cultures for BAC DNA preparation. Using a preculture gives higher reproducibility and more consistent results.

- Grow precultures in a square-well block. Use 150 µl to 1.2 ml of LB or 2 x YT medium with an appropriate antibiotic. Grow with vigorous shaking 300-400 rpm at 37°C for 16 h. Alternatively grow 100 to 150 µl preculture using a standard Microtiter plate (u-bottom) with shaking at 180 rpm. Inoculate precultures with single colonies or glycerol stocks.

Note:

Direct inoculation of cultures for BAC DNA preparation from single colonies or glycerol stocks (without preculture) may result in lower yields and less reproducible results due to higher differences in yields. Use a suitable pin-tool for 96-well plates or 8-channel pipet to inoculate the culture. Avoid repeated freeze/thaw cycles of BAC glycerol stocks. Use replicate plates or prepare fresh glycerol stocks for frequent use.

- Inoculate cultures for BAC DNA peparation from the precultures. Dilute 1 : 1000 (1 µl of preculture per 1 ml of medium with appropriate antibiotic, e.g. 25 µl/ml kanamycin or 12.5 µg/ml chloramphenicol) using a suitable pin-tool or 8-channel pipet.

- When growing BAC cultures in 96-well plates use up to 3 cultures of 1.3 ml per preparation (see ordering information for additional MN Square-well Blocks and Gas-permeable Foils).

- Alternatively, use suitable 48-well deep well blocks (e.g. ABgene 6 ml storage plate, cat. No. AB-0998, also see: www.abgene.com; Whatman Uniplate 48 wells, 5 ml) for culturing BACs. Use 2.5 ml of a suitable culture medium.

- Grow cultures for 16 h at 37°C with shaking. Use the supplied Gas-permeable Foil to cover the culture plate. Discard preculture or prepare new glycerol stocks from preculture.

- Harvest bacteria by centrifugation. Centrifuge culture for 10 min at 2,500 x *g*. When using more then one culture for preparation of DNA from a clone combine corresponding cultures.

- Use a suitable centrifuge rotor with a clearance of 44 mm to accommodate the square well culture plates. Discard medium after centrifugation by inverting the plate quickly. Remove residual culture medium by tapping the plate on a filter paper.

Note:
Please contact our technical service if you need additional information to adapt the NucleoSpin® 96 Flash kit on common laboratory automation workstations as mentioned in section 2.5.

1 Cultivate and harvest bacterial cells

Pellet the bacteria by centrifuging the MN Square-well Block (leave Gas-permeable Foil on the block) at **2,500** × ***g*** for **10 min**. Remove the Gas-permeable Foil and invert the block quickly to pour off the supernatant. Tap the block on a paper sheet or paper towels in order to remove as much of the remaining medium as possible. When growing cultures in several MN Square-well Blocks combine cultures by subsequent centrifugation steps.

2 Resuspend bacterial cells

Add **300 µl F1** to each bacterial pellet and resuspend the cells by vortexing.

Be sure that all of the RNase A has been added to buffer F1 (see section 3). Vortex the block until no more cell clumps or pellets are visible in the wells.

3 Lyse bacterial cells

Add **300 µl F2** to each well, seal the block thoroughly with Self-adhering Foil, and mix by gently inverting the block **10 – 15 times**. Incubate at room temperature for up to **5 min.** ***Do not vortex.*** Remove Self-adhering Foil and dry the upper rim of the wells with a paper towel.

The bacterial suspension should become clear as cell lysis occurs. Vortexing at this step will shear the genomic bacterial DNA, leading to contamination of the resulting plasmid DNA preparation. Do not extend the cell lysis to more than 5 min.

4 Neutralize

Add **300 µl F3** to each well, seal the block with fresh Self-adhering Foil, and mix by gently inverting **10 – 15 times**.

A white precipitate of SDS and cell debris forms.

5 Heat incubation (optional), refer to section 6.1

Prepare the NucleoVac 96 vacuum manifold:

Insert spacers for “Square-well blocks”, notched side up, into the grooves located on the short sides of the manifold. Place a new Square-well Block into the manifold base, close the lid and insert the NucleoSpin® Flash Filter Plate into the lid.

*If using a centrifuge with a swing-bucket rotor capable of hosting 96-well blocks plus the NucleoSpin® Flash Filter Plate (e.g. NucleoSwing Z513), place the NucleoSpin® Flash Filter Plate on a fresh Square-well Block, apply lysates from step 4, and centrifuge for **2 min** at **2,500 × g.** Proceed directly to step 8.*

6 Transfer of crude lysates

Load the cell lysates into the wells of the NucleoSpin® Flash Filter Plate.

7 Clear crude lysates

Apply moderate vacuum (**0.2 – 0.4 bar** reduction of atmospheric pressure) until all of the samples have passed through the column (typically **< 5 min**).

It may be necessary to cover unused wells with a rubber pad if the vacuum does not build up immediately.

8 Precipitate DNA

Remove the Square-well Block from the vacuum manifold. Add **630 µl** (0.7 volumes) of **isopropanol** to each well. Close the block with an Adhesive Foil and mix by inverting the block **6 – 8** times.

Optional: Add 2 mg of glycogen (e.g. Sigma Cat. No G1767) or other carrier for precipitation.

Centrifuge the block for **30 min** at **6,000 × *g* at room temperature**. Use of a refrigerated centrifuge is recommended. For centrifugation at lower g-forces prolonged centrifugation times are required (e.g. 60 min at 2,000 x g).

9 Wash pellet

Remove foil and discard the supernatant by inverting the block quickly. Tap the block on blotting paper or paper towels.

Add **500 µl ice-cold ethanol** (**70%)** per well and seal block again. Centrifuge at **6,000 × *g*** for **10 min**. For centrifugation at lower g-forces prolonged centrifugation times are required (e.g. 15 min at 2,000 x g)

10 Dry pellet

Remove foil and discard the supernatant as described in step 9. Tap the block on paper sheet or paper towels. Dry the pellets at **room temperature** for at least **10 – 15 min** in until **no more ethanol droplets are visible**.

Ethanol is an inhibitor of many enzymatic reactions. Drying of the pellets is also possible at room temperature. However, for optimal removal of ethanol from the preparation, heat incubation is recommended.

11 Resuspend DNA

Add **25-30 µl FE** resuspension buffer (5 mM Tris-HCl, pH 8.5), seal the block with Self-adhering Foil **and incubate the block overnight at room temperature.**

A vortexer or shaker may be used to enhance resuspension when DNA is used for sequencing purposes. Using a vortexer or resuspending by pipetting up and down may cause shearing which might interfere in some mapping applications.

Check the yield and quality of BAC DNA by agarose gel electrophoresis before setting up sequencing reactions: use an aliquot of 5 µl for restriction analysis. Incubate with 5-10 units of an appropriate restriction endonuclease for 1 h. Separate digested BAC DNA on a 0.7 % agarose gel and stain with ethidium bromide. A pattern of distinct fragments should be visible.

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	*Cell pellet not properly resuspended* • It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of lysis buffer F2. If necessary, increase number of mixing cycles or duration of shaking. *SDS in buffer F2 precipitated* • SDS in buffer F2 may precipitate upon storage. If this happens, incubate F2 at 30–40°C for 5 min and mix well. *Too many bacterial cells used* • Usage of LB as the growth medium is recommended. When using rich media like TB or 2 x YT, cultures volumes have to be reduced (see section 5).
Poor plasmid yield	*No or not enough antibiotic used during cultivation* • Cells harbouring the plasmid of interest may become overgrown by nontransformed cells. Add appropriate amounts of freshly prepared stock solutions to all media, solid and liquid. *Bacterial cultures are too old* • See suggestions in section 5 'Growing of bacteria cultures'. *Incomplete lysis of bacterial cells* • See 'Possible cause and suggestions' above.

Problem	Possible cause and suggestions
High level contamination with chromosomal DNA	*Excessive mixing steps after addition of lysis buffers F2 and F3, or before transfer of crude lysate to the NucleoSpin® Flash Filter Plate* • Mix by gentle inversion of the sealed culture block. *Culture volume was too high* • Reduce culture volume if lysate is too viscous for gentle and complete mixing. *Bacterial culture overgrown* • Overgrown bacterial cultures contain lysed cells and degraded DNA. See suggestions in section 5 'Growing of bacteria cultures'. *Lysis was too long* • Lysis step must not exceed 5 min.
Suboptimal performance of plasmid DNA in sequencing reactions, problems with downstream applications	*Resuspension of plasmid DNA with TE buffer* • EDTA may inhibit enzymatic reactions like DNA sequencing. Repurify the plasmid DNA and elute with AE buffer or nuclease-free water. Alternatively, the plasmid DNA may be precipitated with ethanol, and redissolved in FE buffer or nuclease-free water. *Not enough DNA used for sequencing reactions* • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions. *Contamination of final plasmid preparation with ethanol* • Insufficient drying after washing with ethanol, and therefore remaining ethanol may cause problems with downstream applications like DNA sequencing or loading of samples onto agarose gels.

7.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 96 Flash	740 618.2	2 x 96 preps
NucleoSpin® 96 Flash	740 618.4	4 x 96 preps
NucleoSpin® 96 Flash	740 618.24	24 x 96 preps
Square-well Block	740 670	20
MN Square-well Block	740 678	20
Gas-permeable Foil (for cell culture)	740 675	50
NucleoVac 96 vacuum manifold	740 681	1
Vacuum Regulator	740 641	1
Related Products:		
NucleoSpin Robot-96 Plasmid	704 708.2	2 x 96 preps
NucleoSpin Robot-96 Plasmid	704 708.4	4 x 96 preps
NucleoSpin Robot-96 Plasmid	704 708.24	24 x 96 preps
NucleoSpin Multi-96 Plus Plasmid	740 625.1	1 x 96 preps
NucleoSpin Multi-96 Plus Plasmid	740 625.4	4 x 96 preps
NucleoSpin Multi-96 Plus Plasmid	740 625.24	24 x 96 preps

7.3 References

Birnboim, H.C. & Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523.

Vogelstein, B. & Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615-619.

7.4 Product use restriction / warranty

NucleoSpin® 96 Flash kits components were developed, designed and sold **for research purposes only**. They are suitable ***for in vitro uses only***. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).
It is rather in the responsibility of the user to verify the use of the **NucleoSpin® 96 Flash** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL guarantees to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.
Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products.

You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.
Please contact:

MACHEREY-NAGEL Germany
Tel.: +49 (0) 24 21 969 270
e-mail: TECH-BIO@mn-net.com