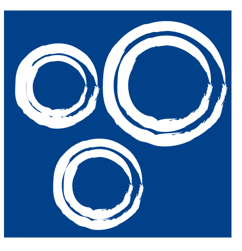

Plasmid and large-construct DNA purification

User manual

NucleoSpin® 96 Flash

April 2014 / Rev. 04

MACHEREY-NAGEL

www.mn-net.com

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1 Components

1.1 Kit contents

	NucleoSpin® 96 Flash		
	2 x 96 preps	**4 x 96 preps**	**24 x 96 preps**[1]
REF	**740618.2**	**740618.4**	**740618.24**
Resuspension Buffer F1	100 mL	250 mL	6 x 250 mL
Lysis Buffer F2	100 mL	250 mL	6 x 250 mL
Neutralization Buffer F3	125 mL	250 mL	6 x 250 mL
Buffer FE[2]	60 mL	60 mL	6 x 60 mL
RNase A (lyophilized)[3]	25 mg	50 mg	6 x 50 mg
NucleoSpin® Flash Filter Plate (dark yelow rings)	2	4	24
Culture plate	2	4	24
Square-well Block (precipitation plate)	2	4	24
Gas-permeable Foil[4]	2	4	24
Self-adhering Foil[5]	10	20	120
User manual	1	1	6

[1] The kit for 24 x 96 preparations (REF 740618.24) consists of 6 x REF 740618.4.

[2] Composition of Buffer FE: 5 mM Tris/HCl, pH 8.5

[3] For preparation of working solutions and storage conditions see section 3.

[4] Every MN Square-well Block (culture plate) includes a Gas-permeable Foil.

[5] For ordering information, please contact Technical Service. Use of Self-adhering PE Foil (REF 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 Buffer 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. Afterwards, all precipitates are 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 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 / HCl, 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 the isolation of DNA from large low-copy DNA such as BACs, see section 5.3.
- The kit is for use under vacuum (common laboratory automation workstations, see section 2.4), NucleoVac 96 Vacuum Manifold (see ordering information) or similar suitable vacuum manifolds (see section 2.3) or in a centrifuge (see section 2.3). 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 Mini preps from *E.coli* cultures with NucleoSpin® 96 Flash is less than 90 min.

Kit specifications at a glance	
Parameter	**NucleoSpin® 96 Flash**
Technology	Alkaline lysis with subsequent filtration and precipitation
Format	96-well plates
Processing	Manual or automated, vacuum or centrifugation (centrifuge required for precipitation)
Lysate clarification	96-well filter plates
Sample material	1.1–1.3 mL *E. coli* culture
Vector size	< 250 kbp
Typical yield	8 µg from 1.1–1.3 mL *E. coli* culture (high-copy plasmid) 1 µg from 1.3–3.9 mL *E. coli* culture (BACs)
Preparation time	90 min/2 plates

2.3 Required hardware

Harvesting of bacterial cells and plasmid precipitation is achieved in a centrifuge with a swinging-bucket rotor attaining ≥ 2,500 x *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 **NucleoSpin® 96 Flash**.

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), no NucleoVac 96 vacuum manifold is necessary and the whole procedure can be performed in the centrifuge. Shorter centrifugation times are sufficient, if the centrifuge is capable of attaining 6,000 x *g*.

2.4 Automated processing on robotic platforms

NucleoSpin® 96 Flash can be used fully automated on many common laboratory workstations. For the availability of scripts and general considerations about adapting **NucleoSpin® 96 Flash** on a certain workstation, please contact MN.

Visit MN online at ***www.mn-net.com*** or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions, and selection of the protocol.

2.5 Growth of bacterial cultures

2.5.1 Selection of culture media

The cultivation of cells is recommended at 37 °C in LB (Luria-Bertani) medium at constant shaking (200–250 rpm). Alternatively, rich media like 2x YT or TB (Terrific Broth) can be used. By using 2x YT or TB, bacteria grow faster and reach the stationary phase much earlier than in LB medium (≤12 h) in culture tubes or flasks. 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.

2.5.2 Cultivation of bacteria in a Square-well Block

Use the 96-well Square-well Block for growing bacteria. Add 1.2–1.5 mL of selected medium (with appropriate antibiotic; for example 100 µg/mL ampicillin) to each well of the 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 Square-well Block with the Gas-permeable Foil supplied with the Square-well Block. Grow the culture in a suitable incubator at 37 °C for 16–24 h with vigorous shaking (200–400 rpm). The 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 such as 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.*

2.5.3 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 2.5 mL 2x YT or 2.5 mL TB medium can be used. Grow bacteria with vigorous shaking (200–250 rpm) for 10–14 h.

Optional: If the liquid handling instrument does not allow for the use of selected culture tubes, transfer the bacterial culture from the tubes into a suitable Square-well Block. Transfer 1.5 mL of the culture to each well of the Square-well Block. Harvest the cultures by centrifugation. Discard supernatant. Usually 1.5 mL of culture are sufficient for DNA preparation. However, if necessary, add additional 1.0–1.5 mL bacterial culture to each well of the Square-well Block, centrifuge again, and discard the supernatant.

Do not use more than 5 mL LB culture or 2.5 mL rapid growing bacterial strain (using 2x YT or TB medium) because lysis efficiency might be lower when using cell pellets which are too large.

2.5.4 Recommendations for the 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 100–150 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 pipette 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 pipette.

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

- Alternatively, use suitable 48-well deep well blocks 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.

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!*

- 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 Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. We recommend a vacuum of 0.2–0.4 bar (reduction of atmospheric pressure). 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 1 mL **Buffer F1** to the **RNase A** vial and vortex. Transfer all of the resulting solution into the Buffer F1 bottle and mix thoroughly. Indicate the date of RNase A addition. Store Buffer F1 containing RNase A at 4 °C. The solution will be stable at this temperature for at least six months.

4 Safety instructions

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
F2	Sodium hydroxide 0.5–2.0 % *Natriumhydroxid-Lösung 0.5–2.0 %*	Warning *Achtung*	290, 315, 319	234, 280, 302+352, 305+351+338, 332+313, 337+313, 390, 406
RNase A	RNase A, lyophilized *RNase A, lyophilisiert*	Danger *Gefahr*	317, 334	261, 280, 302+352, 304+340, 333+313, 342+311, 363

Hazard phrases

H 290 May be corrosive to metals.
Kann gegenüber Metallen korrosiv sein.

H 315 Causes skin irritation.
Verursacht Hautreizungen.

H 317 May cause an allergic skin reaction.
Kann allergische Hautreaktionen verursachen.

H 319 Causes serious eye irritation.
Verursacht schwere Augenreizung.

H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.

Precaution phrases

P 234 Keep only in original container.
Nur im Originalbehälter aufbewahren.

P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.

P 280 Wear protective gloves / eye protection.
Schutzhandschuhe / Augenschutz tragen.

P 302+352 IF ON SKIN: Wash with plenty of water/…
Bei Kontakt mit der Haut: Mit viel Wasser/… waschen.

P 304+340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Bei Einatmen: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.

P 305+351+338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.*
P 332+313	If skin irritation occurs: Get medical advice / attention. *Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 333+313	If skin irritation occurs: Get medical advice / attention. *Bei Hautreizung- oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 337+313	Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*
P 390	Absorb spillage to prevent material damage. *Verschüttete Mengen aufnehmen, um Materialschäden zu vermeiden.*
P 406	Store in a corrosive resistant container with a resistant inner liner. *In korrosionsbeständiger Auskleidung aufbewahren.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com)***.

Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)***.

5 Protocols

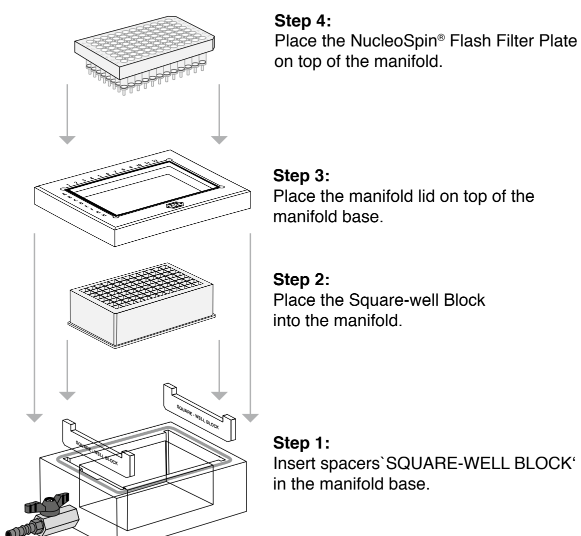
Protocol-at-a-glance

1	**Cultivate** and **harvest** bacterial cells	**1.5 mL–5 mL LB or up to 2.5 mL 2 x YT or TB** **1,000 x *g*, 10 min**
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)**
		Prepare vacuum manifold for lysate clearing step
5	**Heat incubation (optional)**	**100 °C, 5 min**
6	**Transfer** crude lysates to NucleoSpin® Flash Filter Plate	
7	**Clear** crude lysates directly into Square-well Block	**-0.2 to -0.4 bar*, 1–5 min** **or** **1,500 x *g*, 1–5 min**
8	**Precipiate DNA**	**630 µL isopropanol (0.7 volumes)** **≥ 2,500 x *g*, 15 min**

* Reduction of atmospheric pressure

9	**Wash pellet**	**700 µL ethanol (70 %)** **≥ 2,500 x *g*,** **3 min**
10	**Dry pellet**	**55 °C, 10 min**
11	**Resuspend DNA**	**20–50 µL FE**

Setup of vacuum manifold:



5.1 High-copy plasmid purification – manual processing

- For hardware requirements, refer to section 2.3.
- For detailed information regarding the vacuum manifold setup, see page 15.

Before starting the preparation:

- Check if Buffer F1 was prepared according to section 3.

---

1 Cultivate and harvest bacterial cells

Pellet the bacteria by centrifuging the Square-well Block (leave Gas-permeable Foil on the block) at **1,000 x *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 Buffer F1 with RNase A** 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 Buffer 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 Buffer 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 DH5*α *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

Prepare manifold for filtration of crude lysates (see page 15):

Insert spacers labeled 'SQUARE-WELL BLOCK' notched side up into the grooves located on the short sides of the manifold base. 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, 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 x g. Proceed directly to step 8.

6 Transfer crude lysates onto the NucleoSpin® Flash Filter Plate

Transfer the crude lysates resulting carefully and completely into the wells of the NucleoSpin® Flash Filter Plate.

7 Clear crude lysate by vacuum filtration

Apply vacuum of **-0.2 to -0.4 bar*** until all of the samples have passed the wells (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, 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 x g.

8 Precipitate DNA

Remove the Square-well Block from the vacuum manifold. Add **630 µL** (0.7 volumes) **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 x *g***.

9 Wash pellet

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

Add **700 µL ethanol (70 %)** per well and seal block again. Centrifuge at **2,500 x *g*** for **3 min**.

10 Dry pellet

Remove foil and discard the supernatant by inverting the block quickly. 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 Buffer FE** (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 Buffer 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 Mini preps 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.

* Reduction of atmospheric pressure

5.2 High-copy plasmid purification – automated processing

Note: The list numbers in this protocol do not correspond to the numbers in section 6.1.

Required hardware:

- Suitable vacuum manifold

Before starting the preparation:

- Check if Buffer F1 was prepared according to section 3.

---

1 Cultivate and harvest bacterial cells

Pellet the bacteria by centrifuging the Square-well Block (leave gas permeable foil on the block) at **1,000 x *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.

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2 Before placing the 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 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: 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.

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* Reduction of atmospheric pressure

4 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.

5 After addition of **0.7 volumes** of **isopropanol** to each well by the robot, remove the Square-well Block from the vacuum manifold.

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

7 Add **500 µL ethanol (70 %)** per well and seal block again. Centrifuge at **2,500 x *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.

8 Add **50 µL Buffer FE** (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 Buffer 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 Mini preps 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.

5.3 Large low-copy construct DNA purification – automated and manual processing

Please see section 2.5.4 for recommendations regarding the cultivation of large low-copy constructs (e.g., BACs)

Required hardware:

- Suitable vacuum manifold

Before starting the preparation:

- Check if Buffer F1 was prepared according to section 3.

---

1 Cultivate and harvest bacterial cells

Pellet the bacteria by centrifuging the Square-well Block (leave Gas-permeable Foil on the block) at **2,500 x *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 Square-well Blocks, combine cultures by subsequent centrifugation steps.

---

2 Resuspend bacterial cells

Add **300 μL Buffer F1 with RNase A** 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 Buffer 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 Buffer F3** to each well. Seal the block thoroughly with new Self-adhering Foil and mix gently by inverting 10–15 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 DH5*α *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

Prepare manifold for filtration of crude lysates (see page 18):

Insert spacers labeled 'SQUARE-WELL BLOCK' notched side up into the grooves located on the short sides of the manifold base. 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, 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 x g. Proceed directly to step 8.

6 Transfer crude lysates onto the NucleoSpin® Flash Filter Plate

Transfer the crude lysates resulting carefully and completely into the wells of the NucleoSpin® Flash Filter Plate.

7 Clear crude lysate by vacuum filtration

Apply vacuum of **-0.2 to -0.4 bar*** until all of the samples have passed the wells (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 a Self-adhering Foil and mix by inverting the block 6–8 times.

Optional: Add 2 mg of glycogen or other carrier for precipitation.

Centrifuge the block for **30 min** at **6,000 x *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 the foil and discard the supernatant by inverting the block quickly. Tap the block on blotting paper or paper towels.

Add **700 µL ice-cold ethanol (70 %)** per well and seal block again. Centrifuge at **6,000 x *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 an incubator or 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 Buffer FE** (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.

Quantify 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 the digested BAC DNA on an 0.7 % agarose gel and stain with ethidium bromide. A pattern of distinct fragments should be visible.

6 Appendix

6.1 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 Buffer 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 non-transformed 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 Buffer F2 and Buffer 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 Buffer FE or nuclease-free water. *Not enough DNA used for sequencing reactions* • Quantify 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 such as DNA sequencing or loading of samples onto agarose gels.

6.2 Ordering information

Product	REF	Pack of
NucleoSpin® 96 Flash	740618.2 740618.4 740618.24	2 x 96 preps 4 x 96 preps 24 x 96 preps
Square-well Block	740481 740481.24	4 24
Gas-permeable Foil	740675	50
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
NucleoSpin® 96 Plasmid	740625.1 740625.4 740625.24	1 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoSpin® 96 Plasmid Core Kit	740616.4 740616.24	4 x 96 preps 24 x 96 preps

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction/warranty

NucleoSpin® 96 Flash kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

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No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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Last updated: 07/2010, Rev. 03

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