Product List

Product	# of Prep.	Contents	Cat. #
JETSPIN Plasmid Midiprep Kit / 25	25	25 Midi Spin Columns Solutions, Reagents	310025
JETSPIN Plasmid Midiprep Kit / 50	50	50 Midi Spin Columns Solutions, Reagents	310050
JETSPIN Plasmid Midiprep Kit / 100	100	100 Midi Spin Columns Solutions, Reagents	310100
JETSPIN Plasmid Maxiprep Kit / 10	10	10 Maxi Spin Columns Solutions, Reagents	320010
JETSPIN Plasmid Maxiprep Kit / 20	20	20 Maxi Spin Columns Solutions, Reagents	320020
JETSPIN Plasmid Maxiprep Kit / 50	50	50 Maxi Spin Columns Solutions, Reagents	320050

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Protocol

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JETspin

Large-scale Plasmid DNA in Minutes

Description

The **JETSPIN Plasmid Kits** provide a very simple and reliable method to isolate **highly pure plasmid DNA** on the basis of spin columns. The entire procedure is completed in **25 min or less** with **ready-to-use DNA** in TE buffer or water. Time per preparation increases only slightly, when e.g. 12 plasmid preparations are carried out in parallel.
As an **absolute novelty**, the JETSPIN Plasmid Kits are offered with **Midi** or **Maxi JETSPIN Columns** for large-scale plasmid preparations. The average DNA yields are 100 µg for the Midi and 500 µg for the Maxi format of the spin columns.

For the usage of the JETSPIN Plasmid Kits two things must be taken into account:

- **JETSPIN Plasmid Kits are exclusively designed for the preparation of high-copy plasmid DNA.**
- **Swing-out rotors are an absolute requirement.**

Plasmid Purity

The JETSPIN purified plasmid DNA is highly pure, matching CsCl purified plasmid DNA. Its recommended application range is correspondingly extremely wide, including:

- **fluorescent sequencing,**
- **radioactive sequencing,**
- **ligation, cloning,**
- **amplification,**
- **labeling, restriction analysis,**
- **and other enzymatic reactions**

Procedure
The procedure employs a modified alkaline/SDS method to prepare the cleared lysate. After neutralization, the cleared lysate is applied directly onto a **JETSPIN Column** (Midi or Maxi) and the plasmid DNA is bound to the adsorption matrix.
One wash is sufficient to remove RNA, proteins and all other impurities. Afterwards, the purified plasmid DNA is eluted from the spin column. The DNA is ready to use **without a further alcohol precipitation**.
The JETSPIN procedure opens up **a new dimension in the speed** of large scale plasmid isolation, combined with excellent DNA quality.

Culture Volumes

The JETSPIN Plasmid Kits are exclusively designed to extract and purify **high-copy plasmid DNA** from *E.coli* cultures.
GENOMED recommends **LB medium** to grow *E.coli* cells to isolate plasmid DNA with JETSPIN columns. The cell density should be approximately 1 x 10^9 cells per ml LB medium (1-1.5 A_{600} units/ml).

High Copy Plasmids **(2 - 5 µg DNA/ml LB medium)**

Prep Size	Culture Volume	DNA Yield
Midi	up to 25 ml	50-100 µg
Maxi	up to 100 ml	200-500 µg

Description (cont.)

Very important!

Please note that it is not recommended to use larger culture volumes with the standard JETSPIN procedure. This requires a modified protocol, as indicated on pages 8 and 9.

Plasmid Yields

High-copy plasmid DNA of sizes up to 20 kb can be prepared from **up to 25 ml (Midi)** and **up to 100 ml (Maxi)** culture volumes. Yields of **up to 100 µg (Midi) and 500 µg (Midi) of plasmid DNA** can be expected using the JETSPIN Columns.
The yields depend very much on the type of plasmid DNA, the bacterial strain and the volume of bacterial culture used. The recovery of plasmid DNA is on average between 80% and 85%.

Technical Requirements & Reagents to be supplied by the user

- Centrifuge with a swing-out rotor, capable of processing the tube types mentioned below.
- Suitable 50 ml polypropylene tubes to hold the Midi and Maxi spin columns during centrifugations (e.g. from Falcon, Greiner, Sarstedt).
- Ethanol (96-100%)
- TE buffer, 10 mM Tris-HCl (pH 8.0) or water

Solutions

Reconstitution of solution F4

The bottle of solution F4 contains concentrated buffer solution. Before use, add ethanol (96-100%) as stated on the bottle´s label.

Solution F1 **(Cell Suspension)** **Store at 4°C**
50 mM Tris / HCl (pH 8.0)
10 mM EDTA
100 µg/ml RNase A

Solution F2 **(Cell Lysis)** **Store at RT**
200 mM NaOH
1 % SDS (w/v)

Solution F3 **(Neutralization/Binding)** **Store at RT**
Contains acetate and guanidine hydrochloride

Solution F4 **(Wash, reconstituted)** **Store at RT**
Contains ethanol, NaCl, EDTA and Tris/HCl

TE buffer **(DNA Elution)** **Store at RT**
10 mM Tris / HCl (pH 8.0)
0.1 mM EDTA

(TE buffer is not supplied with the kit)

RT = room temperature

Protocol for JETSPIN Midi and Maxi

Very important!

- **Before starting the procedure, make sure that solution F4 is reconstituted, as indicated on the bottle´s label.**
- **All steps are performed at room temperature.**
- **Swing-out rotors are an absolute requirement.**

1. **Harvesting Bacterial Cells** *E. coli* cells are pelleted by centrifugation. Remove **all** traces of medium carefully.

2. **Cell Resuspending** Add **solution F1** to the pellet and re-suspend the cells until the suspension is **homogeneous**:

   Solution F1 / Midi: 4.0 ml
   Maxi: 6.0 ml

3. **Cell Lysis** Add **solution F2** and mix gently by inverting the tube several times until a homogeneous mixture is obtained. **Do not vortex!** Incubate at **room temp.** for **5 min**.

   Solution F2 / Midi: 4.0 ml
   Maxi: 6.0 ml

4. **Neutralization** Add **solution F3** and mix **immediately**, but gently, by inverting the tube several times until a homogeneous suspension is obtained. **Do not vortex!** Centrifuge the mixture at **room temperature** and **≥12.000 x g** for **15 min**.

   Solution F3 / Midi: 6.0 ml
   Maxi: 8.0 ml

5. **Column Loading** Place the spin column into a 50 ml receptacle (i.e. Falcon tube). Load the supernatant from step 4 into the prepared spin column. Centrifuge at **5.000 x g** for **2 min** in a **swing-out rotor**.

6. **Column Washing** Discard the flow-through. Re-assemble the spin column with the receptacle and add **solution F4** to the **empty** spin column. Centrifuge at **5.000 x g** for **2 min** in a **swing-out rotor**. Discard the flowthrough, re-assemble the Maxi-Spin unit with the receiver tube and centrifuge for **5 min at 5.000 x g** to clear the membrane from residual buffer F4.

   Solution F4 / Midi: 10.0 ml
   Maxi: 15.0 ml

7. **Plasmid Elution** Transfer the spin column into a new, clean tube. Elute the DNA with **TE buffer** (not supplied with the kit) or **water** (for plasmids larger 10 kb, preheat TE buffer or water to 70°C). Centrifuge at **5.000 x g** for **2 min** in a **swing-out rotor.**

   TE or water / Midi: 0.8 ml
   Maxi: 3.0 ml

Depending on the later usage of the plasmid DNA, the elution volume can be varied. The data listed below indicate the correlation between added volume of TE buffer or water and the recovery of plasmid DNA in relationship to 100% recovery (MAXI prep).

Volume	Recovery
1.0 ml	55%
1.5 ml	65%
2.0 ml	75%
2.5 ml	79%

Volume	Recovery
3.0 ml	89%
4.0 ml	92%
6.0 ml	95%
8.0 ml	98%

Comments on the Protocol

<u>Handling of spin columns</u>

Centrifugation of spin columns must be carried out in a swing-out rotor. If not, the efficiency of the DNA binding, washing and elution step will be poor and the DNA yields will be low, as well as contaminations of the plasmid DNA with RNA, protein, salt or ethanol possibly occuring.

Comments on step 1:

- Make sure that culture medium back-draining from the tube´s wall is removed. Last traces of medium should be removed with a pipette.
- **Culture volumes larger than those recommended will result in poor DNA recovery and poor DNA quality.**
- For the processing of larger culture volumes, see the modified protocol on page 8 and 9.

Comments on step 3:

- If SDS is precipitated in solution F2 due to low temperatures during transport or storage of the product, redissolve it by warming solution F2 in a water bath for a few minutes.
- To avoid shearing of the genomic DNA, do not vortex the mixture. The incubation following should not be proceeded with for more than 5 min, otherwise plasmid DNA might be denaturated irreversibly.

Comments on step 4:

- The centrifugation to pellet the bacterial debris is performed best when the mixture is homogeneous. Thus, mix gently by inverting the tube several times immediately after addition of solution F3. Do not vortex!
- When many samples are prepared in parallel, each sample should be mixed immediately after adding solution F3.

Comments on step 5:

- After centrifugation save the supernatant with a pipette, and avoid getting too many particles into the transferred supernatant.

Comments on step 6:

- The prolonged centrifugation time is necessary for the quantitative removal of ethanol.

Trouble-Shooting Guide

Please note that by not adhering to the protocol unsatisfactory results regarding yield and quality of the plasmid DNA may occur! If problems arise, check the following points:

1. All conditions regarding volumes, temperatures, incubation times and centrifugations were carried out precisely.

2. The solutions were stored at recommended temperatures.

3. JETSPIN Columns were not overloaded with plasmid DNA. The recommended culture volumes and calculated amounts of plasmid DNA have been taken into account.

Low yields of plasmid DNA!

1. The total amount of plasmids in *E. coli* cells is very much dependent on the individual host-plasmid system. Plasmids vary in their copy number per cell (low/medium/high). The range of plasmid DNA per ml culture can vary from 0.2 µg/ml (low copy) to >5.0 µg/ml (high copy). Additionally, the size and sequence of specific DNA inserts influence the copy number of a particular plasmid, and so the yield of plasmid DNA.

2. It is important that the specified volumes of solutions F1-F3 are kept precise. Otherwise the required balance between F1, F2 and F3 will be destroyed. This may also occur, if back-draining culture medium is present or a surplus of solution F3 is added for neutralization.

Low plasmid contents in bacteria!

If the host cells contain only low amounts of plasmid DNA, **DO NOT increase the culture volume** (and so the amount of bacteria to be lysed) beyond the recommended limits (see page 2). In this case, a severe decrease in the DNA yield may be observed be-

cause of an incomplete lysis procedure. This can be circumvented by the following modification of the standard procedure:

1. Resuspend the bacterial pellet from up to 100 ml culture volume for Midi in 4 ml and from up to 500 ml culture volume for Maxi in 10 ml of the Cell Suspension Solution (solution F1).

2. Add 4 ml (Midi) or 10 ml (Maxi) of Cell Lysis Solution (solution F2), mix and incubate, according to step 3 of the standard proto col.

3. Add 4 ml (Midi) or 10 ml (Maxi) of a 3.0 M potassium acetate solution (pH 5.0; **not** supplied with the kit), mix and centrifuge, according to step 4 of the standard protocol.

4. Save the supernatant and precipitate the nucleic acids with 8 ml (Midi) or 20 ml (Maxi) isopropanol. Centrifuge at >12.000 x g for 15 min, discard the supernatant and redissolve the pellet in 8 ml (Midi) / 12 ml (Maxi) TE buffer.

5. Add 6 ml (Midi) / 8 ml (Maxi) of solution F3 and proceed with **step 5** of the standard protocol.

DNA concentration too low!

Should the DNA concentration in the eluate be too low, the elution volume can be reduced (see table on page 6 of the protocol). Smaller elution volumes lead to higher DNA concentrations (µg DNA / ml), but the absolute yield of DNA does decrease.
Alternatively, the eluted DNA (standard elution volumes are 0.8 ml for Midi and 3 ml for Maxi) can be concentrated by a common alcohol precipitation.

Chromosomal DNA contamination!

Chromosomal bacterial DNA is removed from the preparation by precipitation, after the addition of solution F3 and the centrifugation that follows. This is only successful if shearing of the chromosomal DNA after cell lysis is kept to a minimum. Shearing of the chromosomal DNA occurs when the sample is vortexed, after the addition of solution F2 or solution F3. **Avoid vortexing during steps 3 and 4 of the protocol!**

Additional plasmid forms!

An additional plasmid form, running on agarose gels in front of the supercoiled form, is due to irreversible denaturated plasmid DNA. The irreversible denaturation is probable if the cell lysis (protocol, step 3) was carried out for longer than the recommended 5 min under the strongly alkaline conditions.

RNA contamination!

RNase A digestion was insufficient. Make sure that culture volumes used correspond to those recommended (see table on page 2). **If solution F1 (contains RNase) is older than 6 months, add new RNase.**

Ethanol in the eluate!

Centrifugation time was too short (step 6 of the protocol) or a fixed-angle rotor was used.

Spin column destroyed!

Check rpm-value. The resulting centrifugal force should not exceed 5.000 x g. Check whether centrifugation tube and spin column fit together. The buckets should swing freely in the rotor.