

Product List

Product	Prep.	Contents	Cat. #
JETQUICK Plasmid Miniprep Spin Kit 50	50	50 Spin Columns Solutions, Reagents	400050
JETQUICK Plasmid Miniprep Spin Kit 250	250	250 Spin Columns Solutions, Reagents	400250
JETQUICK Gel Extraction Spin Kit 50	50	50 Spin Columns Solutions, Reagents	420050
JETQUICK Gel Extraction Spin Kit 250	250	250 Spin Columns Solutions, Reagents	420250
JETQUICK PCR Purification Spin Kit 50	50	50 Spin Columns Solutions, Reagents	410050
JETQUICK PCR Purification Spin Kit 250	250	250 Spin Columns Solutions, Reagents	410250

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Protocol

March 2005

JETquick

Spin Column Technique

Plasmid Miniprep

Gel Extraction

PCR Purification



New Version!!!

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JETQUICK Plasmid Miniprep Spin Kit

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JETQUICK Gel Extraction Spin Kit

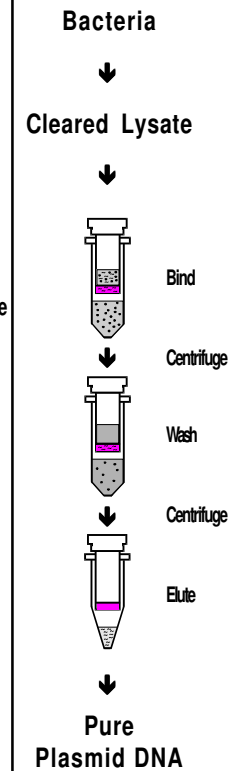
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JETQUICK PCR Purification Spin Kit

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Description / Plasmid Miniprep

Procedure:	< 20 min
DNA Yield:	up to <u>50 µg</u>
DNA Recovery:	90 - 95%
DNA Sizes:	up to 20 kb
DNA Purity:	matches CsCl
DNA Elution:	buffer or water
Advantages:	No phenol No chloroform A260/A280 > 1.8 1 - 5 ml culture volume high reproducibility



Description / Plasmid Miniprep

The **JETQUICK Plasmid Miniprep Spin Kit** provides a very simple and reliable method to isolate **highly pure plasmid DNA** on the basis of spin columns. The entire procedure is completed in **20 min or less** with **ready-to-use DNA** in TE buffer or water.

IMPORTANT!

The **JETQUICK Plasmid Miniprep Spin Kit** is preferably intended for the preparation of **high copy plasmid DNA**.

Procedure: The procedure employs a modified alkaline/SDS method to prepare the cleared lysate. After neutralization, the cleared lysate is applied directly onto a **JETQUICK Micro-Spin Column** and the plasmid DNA is bound to the adsorption matrix.

One wash is usually sufficient to remove RNA, proteins and all other impurities. The purified plasmid DNA is eluted from the spin column in TE buffer or water (**no alcohol precipitation**).

Plasmid yields: High copy plasmid DNA of sizes up to 20 kb can be prepared from **up to 5 ml culture volumes**. Yields of **up to 50 µg of plasmid DNA** can be expected using the JETQUICK columns. Yield depends 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 90% and 95%.

Plasmid purity: The **JETQUICK**-purified plasmid DNA is highly pure matching CsCl purified plasmid DNA. Its recommended application range is correspondingly extremely wide, including **fluorescent and radioactive sequencing, ligation, cloning, amplification, labeling, restriction analysis, and other enzymatic reactions**.

Culture Volumes and Solutions

Culture Volumes The **JETQUICK Plasmid Kit** is preferably intended 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 JETQUICK columns. However, the JETQUICK system is compatible with other growth media as well, **especially media designed for enhanced plasmid yields**. The cell density should be approximately **1 x 10⁹ cells per ml medium** (1-1.5 A₆₀₀ units/ml).

Reconstitution of buffers GX and G4

The bottles of buffers **GX** and **G4** contain concentrated buffer solution. Before use, add absolute ethanol (96-100%) as stated on the bottle's label.

Solution G1 (Cell Suspension) 50 mM Tris/HCl (pH 8.0); 10 mM EDTA; 100 µg/ml RNase A	Store at 4 °C
Solution G2 (Cell Lysis) 200 mM NaOH; 1% SDS (w/v)	Store at RT
Solution G3 (Neutralization/Binding) Contains acetate and guanidine hydrochloride	Store at RT
Solution GX (Wash, optional) Contains guanidine hydrochloride	Store at RT
Solution G4 (Column Wash) Contains ethanol, NaCl, EDTA and Tris-HCl	Store at RT

RT = room temperature

Protocol / Plasmid Miniprep

Very important!

- Before starting the procedure, make sure that **buffers GX and G4 are reconstituted**, as indicated on the bottle's label.
- **Solution G1** contains RNase and should be stored at 4°C.
- All centrifugation steps are carried out at $\geq 12,000 \times g$ in a conventional table-top microfuge.
- **Do not overload** the spin column. The use of 1-3 ml *E.coli* cultures is recommended. The maximum of culture used should be 5 ml. With higher culture volumes DNA yield and quality may decrease rapidly.

1. Harvesting Bacterial Cells *E. coli* cells are pelleted by centrifugation. Remove all traces of medium carefully. Make sure that culture medium back-draining from the tube's wall is removed.

2. Cell Resuspending Add **250 µl** of solution **G1** to the pellet and resuspend the cells (by vortexing or with a pipette) until the suspension is **homogeneous**.

3. Cell Lysis Add **250 µl** of solution **G2** and mix gently, but thoroughly, by inverting the tube several times. **Do not vortex!** Incubate at **room temperature** for **5 min**.

4. Neutralization Add **350 µl** of solution **G3** and mix **gently** but **thoroughly**, by inverting the tube until a **homogeneous** suspension is obtained. **Do not vortex!** Centrifuge the mixture at **room temp.** and at **maximum speed** for **10 min**.

Protocol / Plasmid Miniprep

5. Column Loading Place a **JETQUICK spin column** into a 2 ml receiver tube (provided). Load the supernatant from step 4 into the spin column. Centrifuge at **>12.000 x g** for **1 min**. Discard the flowthrough.

6. (Optional) This additional column wash is recommended, if nuclease-rich bacteria (e.g. *endA*⁺ strains of *E. coli*) are processed:
After having emptied the receiver tube re-insert the micro-spin column into it. Add **500 µl** of **reconstituted buffer GX** into the spin column and centrifuge at **>12.000 x g** for **1 min**. Discard flowthrough and place the **JETQUICK** column back into the same receiver tube. Proceed with step 7.

7. Column Washing Empty the receiver tube, and re-insert the spin column into the receiver tube. Add **500 µl** of **reconstituted buffer G4** and centrifuge at **>12.000 x g** for **1 min**. Discard flowthrough and place the spin column back into the same receiver tube. Centrifuge **again** at **maximum speed** for **1 min**.
Important: Residual solution G4 will not be completely removed unless the flowthrough is discarded before this additional centrifugation.

8. Plasmid Elution Place the **JETQUICK spin column** into a new 1.5 ml microfuge tube and add **75 µl** of **sterile water** (or **TE buffer** or **10 mM Tris/HCl, pH 8**) directly onto the center of the silica matrix of the spin column. Centrifuge at **>12.000 x g** for **2 min**.

Important: Higher DNA concentrations can be obtained if the elution is carried out in only 50 µl elution buffer volume. In this case, preheat your elution buffer to 65-70°C, add the buffer onto the center of the silica matrix of the spin column and let stand for 1 min before centrifugation. Preheated elution buffer is generally recommended when plasmids >5 kb are eluted. DNA eluted in water should be stored at -20°C.

Trouble-Shooting/Plasmid Miniprep

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. JETQUICK columns were not overloaded with *E. coli* culture. The recommended culture volumes and calculated amounts of plasmid DNA have been taken into account.
4. Buffers GX and G4 have been reconstituted with absolute ethanol.

Low yields of plasmid DNA!

1. The total amount of plasmids in *E. coli* cells depends very much 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 more than 5.0 µg/ml (high copy). Additionally, the size and sequence of specific DNA inserts may 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 G1-G3 are kept precisely.

Low plasmid contents in bacteria!

If the host cells contain only low amounts of plasmid DNA, it is **not recommended to increase the culture volume** and the amount of bacteria beyond the recommended limits. In such a case a severe decrease of DNA yield may be observed, because of incomplete bacterial lysis.

Trouble-Shooting/Plasmid Miniprep

DNA concentration too low!

If the DNA concentration in the eluate is too low, the volume of elution buffer can be reduced. Smaller elution volumes lead to higher DNA concentrations (µg DNA/ml), but the absolute yield of DNA does decrease.

Alternatively, the eluted DNA 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 G3 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 G2 or solution G3.

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 denatured plasmid DNA. The irreversible denaturation is likely to occur if the cell lysis (protocol, step 3) has been carried out for longer than the recommended 5 min under strongly alkaline conditions.

RNA contamination!

RNase A digestion was insufficient. Make sure that culture volumes used correspond to those recommended. **If solution G1 (contains RNase) is older than 6 months, add new RNase.**

Ethanol in the eluate!

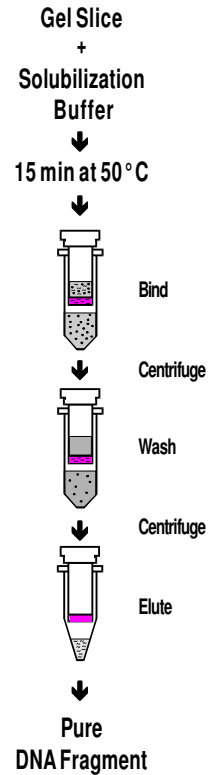
Centrifugation time was too short (step 7 of the protocol).

Need more information?

Check out our website www.genomed-dna.com under 'Troubleshooting'.

Description / Gel Extraction

Procedure: 25 min
DNA Yield: up to 20 µg dsDNA
DNA Recovery: 85-95%
DNA Sizes: 40 bp-10 kb
DNA Purity: highly pure
DNA Elution: buffer or water
Advantages: No phenol
No chloroform
Extraction from:
- high - melt agarose
- agarose > 2%
- TAE, TBE, TPE gels



Solutions / Gel Extraction

Reconstitution of buffer L2

The bottle of buffer L2 contains concentrated buffer solution. Before use, add absolute ethanol (96-100%) as stated on the bottle's label.

Solution L1 (Gel Solubilization) Store at RT
Contains NaClO₄, sodium acetate and TBE-solubilizer

Solution L2 (Wash, reconstituted) Store at RT
Contains ethanol, NaCl, EDTA and Tris/HCl

RT = room temperature

L1 contains sodium perchlorate, use with proper precaution.

Protocol / Gel Extraction

Very important!

- Before starting the procedure, make sure that solution L2 is reconstituted, as indicated on the bottle's label.
- All centrifugation steps are carried out at $\geq 12,000 \times g$ in a conventional table-top microfuge.
- The use of 100 mg agarose gel slices per extraction is recommended (highest DNA purity). The maximum weight of gel slices is 400 mg per extraction.

1. Solubilization of agarose Excise the agarose gel slice (high or low melting point agarose can be used) and transfer the slice into a suitable tube (don't use glass tubes at this step).

For each 100 mg gel slice add 300 μl of solution L1 and incubate at 50°C for 15 min. Mix every 3 min by flicking or vortexing the tube to ensure that the agarose is completely solubilized.

***Important:** For gel slices with > 2% agarose concentrations, add 600 μl of solution L1 per 100 mg gel weight instead of 300 μl . Larger gel slices ($\geq 300 \text{ mg}$) and gel slices of higher agarose concentrations ($\geq 2\%$) take slightly longer to dissolve. In these cases increase the incubation time to 20 - 30 min. Cutting larger gel slices into small pieces will enhance solubilization. The maximum amount of agarose gel per JETQUICK column is 400 mg. **Do not overload** the JETQUICK columns.*

2. Column loading Place a JETQUICK spin column into a 2 ml receiver tube and load the mixture from step 1 into the prepared spin column. Centrifuge at $> 12,000 \times g$ for 1 min. Discard the flowthrough. **The spin column can be loaded with 600 μl .** For larger sample volumes multiple loadings are necessary.

Protocol / Gel Extraction

***Important:** If the weight of the initial agarose gel slice is $> 250 \text{ mg}$, it is recommended to insert an additional washing step with solution L1. Therefore, add 500 μl of solution L1 to the JETQUICK column, let stand for 1 min at room temperature and centrifuge at $> 12,000 \times g$ for 1 min. This additional step will yield better results if the eluted DNA is subsequently used for direct sequencing, in vitro transcription or microinjection.*

3. Column washing Re-insert the spin column into the receiver tube and add 500 μl of the reconstituted solution L2 into the spin column. Centrifuge at $> 12,000 \times g$ for 1 min. Discard the flowthrough. Place the JETQUICK column back into the same receiver tube, and centrifuge again at maximum speed for 1 min.

***Important:** Residual solution L2 will not be completely removed unless the flowthrough is discarded before this additional centrifugation. The additional centrifugation step assures that no residual ethanol will be carried over into the next step.*

4. DNA Elution Place the JETQUICK spin column into a new 1.5 ml microfuge tube and add 50 μl of sterile water (or TE buffer or 10 mM Tris-HCl, pH 8.0) directly onto the center of the silica matrix of the JETQUICK spin column. Centrifuge at $> 12,000 \times g$ for 2 min.

***Important:** Higher DNA concentrations can be obtained if the elution is carried out with only 30 μl of elution buffer. In this case, preheat the elution buffer to 65-70°C, add the buffer directly onto the center of the spin column and let stand for 1 min before centrifugation. Ensure that the elution buffer is dispensed directly onto the silica membrane. Preheated elution buffer is generally recommended to achieve an optimal DNA elution. DNA eluted in water should be stored at -20°C.*

Trouble-Shooting / Gel Extraction

When yields are low!

The protocol is designed to achieve high yields of more than 80%, depending on the size and amount of the DNA to be extracted. If low yields occur, this might be associated with:

- 1. Incorrect ratio of gel slice to solution L1:**
 - see step 1 of the protocol
- 2. Incomplete solubilization of the gel slice:**
 - see step 1 of the protocol
- 3. Incomplete DNA elution:**
 - see step 4 of the protocol
 - preheat the elution buffer to 65 - 70°C, add the buffer directly onto the center of the silica matrix of the spin column and let stand for 1 min. Centrifuge at maximum speed for 1 min.
- 4. For large DNA fragments (> 5 kb):**
 - preheat the elution buffer to 65 - 70°C, add the buffer directly onto the center of the silica matrix of the spin column and let stand for 1 min. Centrifuge at maximum speed for 1 min.
- 5. Supercoiled or circular DNA (plasmid DNA):**
 - the kit is **not** designed to extract supercoiled plasmid DNA from agarose gels.

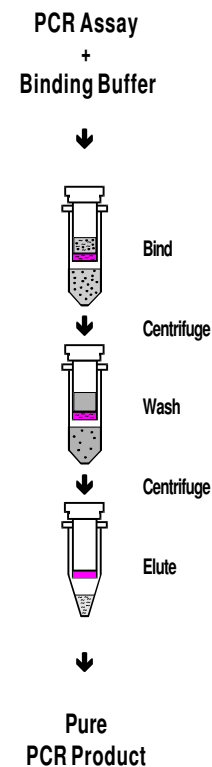
When enzymatic reactions are inhibited!

After elution the DNA is ready-to-use for many applications. Unsatisfactory results can be caused by the following:

- 1. Residual ethanol in the sample after DNA elution:**
 - see step 3 of the protocol
- 2. Residual salt in the sample after DNA elution:**
 - see step 3 of the protocol
 - use water or the following buffer to elute your DNA: (10 mM Tris/HCl, pH 8.0 with **no** EDTA)

Description / PCR Purification

Procedure: 5 min
DNA Yield: up to 20 µg dsDNA
DNA Recovery: 85-95%
DNA Sizes: 80 bp - 20 kb
DNA Purity: highly pure
DNA Elution: buffer or water
Advantages: No phenol
No chloroform
No mineral oil removal
Primer removal >99.8%
Separates 100bp dsDNA from 40mer primers



Solutions / PCR Purification

Reconstitution of buffer H2

The bottle of buffer H2 contains concentrated buffer solution. Before use, add absolute ethanol (96-100%) as stated on the bottle's label.

Solution H1 (Binding) Store at RT
Contains guanidine hydrochloride, isopropanol

Solution H2 (Wash, reconstituted) Store at RT
Contains ethanol, NaCl, EDTA and Tris/HCl

RT = room temperature

H1 contains guanidine hydrochloride, use with proper precaution.

Protocol / PCR Purification

Very important!

- Before starting the procedure, make sure that solution H2 is reconstituted, as indicated on the bottle's label.
- Removal of mineral oil is not required.
- The JETQUICK procedure is not sensitive to detergents (like Triton X-100, NP40) or gelatin in the assay. Nevertheless, concentrations > 0.1% of Tween 20 should be avoided.
- All centrifugation steps are carried out at $\geq 12,000 \times g$ in a conventional table-top microfuge.

1. **Sample Preparation** Add **400 μ l** of solution **H1** to **100 μ l PCR assay** and mix thoroughly.

***Important:** Make sure that the volume of your PCR assay does not exceed 100 μ l (not including the mineral oil overlayer). When **PCR assays >100 μ l are used**, remove most of the mineral oil with a pipette, and scale up solution H1 proportionally. In this case, multiple loadings of the spin column (step 2 of the protocol are required).*

2. **Column Loading** Place a **JETQUICK spin column** into a 2 ml receiver tube. Load the mixture from step 1 into the prepared spin column. Centrifuge at **>12,000 $\times g$** for **1 min**. Discard the flowthrough.

Protocol / PCR Purification

3. Column Washing Re-insert the spin column into the empty receiver tube and add **500 µl of reconstituted solution H2**. Centrifuge at **>12,000 x g** for **1 min**.

Discard flowthrough and place the JETQUICK column back into the same receiver tube. Centrifuge **again at maximum speed for 1 min**.

Important: Residual solution H2 will not be completely removed unless the flowthrough is discarded before this additional centrifugation. Solution H2 contains ethanol, and residual ethanol may interfere with subsequent reactions. The additional centrifugation assures that no residual ethanol is carried over into the next step of the protocol.

4. DNA Elution Place the **JETQUICK spin column** into a new 1.5 ml microfuge tube and add **50 µl of sterile water** (or **TE buffer** or **10 mM Tris/HCl, pH 8.0**) directly onto the center of the silica matrix of the JETQUICK spin column. Centrifuge at **>12,000 x g** for **2 min**.

Important: Higher DNA concentrations can be obtained if the elution is carried out with only 30 µl of elution buffer. In this case preheat the elution buffer to 65-70 °C, add the buffer directly onto the center of the silica matrix of the spin column and let stand for 1 min before centrifugation. Make sure, that the elution buffer is dispensed directly onto the silica membrane. Preheated elution buffer is generally recommended when PCR fragments >5 kb are eluted. DNA eluted in water should be stored at -20 °C.

Trouble-Shooting / PCR Purification

When yields are low!

The protocol is designed to achieve high yields of more than 80%. If low yields occur, they might be associated with:

- 1. Insufficient amplification:**
 - Make sure that your PCR assay has worked well. Apply an aliquot of the PCR assay onto an agarose gel and check the amount of the desired PCR product.
- 2. Incorrect DNA binding conditions:**
 - Make sure that the ratio of solution H1 (400 µl) to your PCR assay (up to 100 µl) was correct.
- 3. Incomplete DNA elution:**
 - see step 4 of the protocol
 - preheat your elution buffer to 65 - 70 °C, add the buffer directly onto the center of the silica matrix of the spin column and let stand for 1 min. Centrifuge at maximum speed for 1 min.
- 4. For large DNA fragments (> 5 kb):**
 - preheat your elution buffer to 65 - 70 °C, add the buffer directly onto the center of the silica matrix of the spin column and let stand for 1 min. Centrifuge at maximum speed for 1 min.

When enzymatic reactions are inhibited!

After elution the DNA is ready-to-use for many applications. Unsatisfactory results can be caused by the following:

- 1. Residual ethanol in the sample after DNA elution:**
 - see step 3 of the protocol
- 2. Residual salt in the sample after DNA elution:**
 - see step 3 of the protocol
 - use water or the following buffer to elute your DNA: (10 mM Tris/HCl, pH 8.0 with **no** EDTA)