**Product List**

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<th>Product</th>
<th>Prep.</th>
<th>Contents</th>
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<td>JETPREP Plasmid Miniprep Kit / 50</td>
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<td>Solution D4 (JETPREP) , Solutions D1, D2, D3, D5</td>
<td>140050</td>
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<td>JETPREP Plasmid Miniprep Kit / 100</td>
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**Worldwide Contact:**
GENOMED GmbH  
Poststrasse 22  
D-32584 Löhne  
Tel: (49)-(0)5732-90470-0  
Fax: (49)-(0)5732-90470-10  
E-mail: techservice-genomed@gmx.net  
Web: www.genomed-dna.com
**Description**

**JETPREP Plasmid Miniprep Kit**
The extraction and purification of plasmid DNA from *E. coli* cells is one of the most tedious and time-consuming, but nevertheless necessary procedures in molecular biology. This is especially true when large numbers of small-scale plasmid preparations (minipreps) are required. A number of techniques to process minipreps are available, but many of them have disadvantages regarding quality of the extracted plasmid DNA, convenience and speed of the procedure or costs per preparation. Bearing all these disadvantages in mind, GENOMED has developed a **Plasmid Miniprep Kit**, which provides a simple, reliable and cost-saving method to isolate plasmid DNA.

**Culture volumes:** The JETPREP Plasmid Miniprep Kit is designed to extract and purify plasmid DNA from 1.0 - 3.0 ml *E. coli* cultures. Frozen cells or fresh cultures can be used.

**Procedure:** The procedure employs a standard alkaline method to lyse the bacterial cells. After neutralization, the plasmid DNA is selectively bound to the JETPREP resin in the presence of guanidine-HCl. DNA binding is completed within 5 min. The JETPREP resin is collected by a short centrifugation. Two washes of the resin with low salt buffer remove contaminants and impurities, such as RNA and proteins. Afterwards, the resin is dried for a few minutes and the plasmid DNA can be eluted in a small, variable volume of water or TE buffer (see protocol).

**Plasmid purity:** The purified plasmid DNA is free of RNA, proteins, salts and other macromolecular contaminants. No organic extraction or ethanol precipitation is required during or after the procedure. The purified plasmid DNA can be used directly for DNA sequencing, restriction digestion, cloning and other common techniques.

**Plasmid yields:** A yield of up to 12 µg of plasmid DNA can be expected using the JETPREP Miniprep System. The yield will vary depending on the copy numbers and the type of the plasmid, the bacterial strain and the volume of bacterial culture used. The recovery of plasmid DNA is between 80% and 90% on average. All types of plasmid DNA can be isolated, but yields depend very much on the copy number (low/medium/high) and the size of the plasmid. Larger plasmids (> 10 kb) can be isolated without changing the protocol, but give a slightly lower yield.

**Storage/Stability:** All JETPREP Miniprep solutions (see below) are guaranteed to be stable for at least six months (from the date of purchase) when stored at room temperature. Protect solutions from direct sunlight.

**Store all solutions at room temperature!**

**Reconstitution of solution D5:** The bottle of solution D5 contains concentrated buffer solution. Before use, add ethanol (95-100% ethanol) as indicated on the bottle’s label.

**Solution D1 (Cell Suspension Solution)**
50mM Tris/HCl (pH 8.0), 10mM EDTA, 100µg/ml RNase A

**Solution D2 (Cell Lysis Solution)**
0.2M NaOH, 1% SDS

**Solution D3 (Neutralization Solution)**
Concentrated acetate (pH 5.0)

**Solution D4 (DNA Binding Solution) Shake well before use!**
JETPREP resin in concentrated guanidine-HCl

**Solution D5 (Wash Solution, reconstituted)**
10mM Tris/HCl (pH 7.5), 50mM NaCl, 0.1mM EDTA, 70% ethanol
Before you start the procedure, make sure that solution D5 is reconstituted (as indicated on the bottle’s label).

1. **Harvesting bacterial cells**
   Bacterial cells from a 1-3 ml overnight culture are pelleted by centrifugation (10,000 rpm; 1 min), and the supernatant is removed completely. Make sure that culture medium back-draining from the wall of the tube is removed.

2. **Cell Resuspending**
   Add 200 µl of solution D1 to the pellet. Resuspend the cells with a pipette or by vortexing until the suspension is homogeneous. No lumpy cells should be visible.

3. **Cell Lysis**
   Add 200 µl of solution D2 and mix gently by inverting the tube several times. Do not vortex! Incubate the mixture at room temperature for 5 min.

4. **Neutralization**
   Add 200 µl of solution D3 and mix thoroughly but gently by inverting the tube several times. Do not vortex! Centrifuge the mixture in a minifuge at room temperature (unchilled minifuge) or 4°C and >10,000 rpm for 10 min. When many samples are prepared in parallel, add solution D3 to the first sample and mix gently before you continue with the second sample.

5. **DNA Binding**
   (Shake solution D4 well immediately before use!)
   After centrifugation, transfer the supernatant with a pipette into a fresh tube immediately. Avoid transferring portions of the precipitate. Add 500 µl of solution D4 (solution D4 contains the JETPREP resin), mix gently and incubate at room temperature for 5 min. Mix once during incubation.

6. **DNA Washing**
   (Reconstitute solution D5 before use!)
   Centrifuge in a minifuge (>10,000 rpm; at room temperature for 30 sec) and remove the supernatant from the pelleted resin completely with a pipette. Wash (resuspend) the pellet with 600 µl of reconstituted solution D5. Collect the resin by centrifugation as before (>10,000 rpm; at room temperature for 30 sec) and remove the supernatant with a pipette quantitatively. Repeat this washing step once! It is extremely important to remove the last supernatant quantitatively to avoid long drying times before DNA elution (see step 7).

7. **Plasmid DNA Elution**
   Dry the JETPREP resin under vacuum until it turns white (using a speed-vac approx. 1-2 min, using a vacuum chamber with a Water Jet Filter Pump approx. 5-7 min) For DNA elution, as a minimal volume add 30 µl TE buffer (pH 7-8) or water. Resuspend the pellet carefully by flicking the tube and incubate for 5 min at 60°C. Flick the tube once during incubation. Centrifuge as before and transfer the supernatant immediately to a new tube. Avoid having too many beads of the resin in the transferred eluate. Now the plasmid DNA is ready to use!

   Depending on the later usage of the plasmid DNA, the elution volume can be varied. The data listed below indicate the correlations between added volume of TE buffer or water, the recovered volume after DNA elution and the recovery of plasmid DNA in relationship to optimal elution conditions.

<table>
<thead>
<tr>
<th>Added volume</th>
<th>Recovered volume</th>
<th>Plasmid Recovery</th>
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<tbody>
<tr>
<td>30 µl</td>
<td>approx. 22 µl</td>
<td>approx. 78%</td>
</tr>
<tr>
<td>50 µl</td>
<td>approx. 42 µl</td>
<td>approx. 86%</td>
</tr>
<tr>
<td>70 µl</td>
<td>approx. 62 µl</td>
<td>approx. 89%</td>
</tr>
<tr>
<td>90 µl</td>
<td>approx. 82 µl</td>
<td>approx. 93%</td>
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Trouble-Shooting Guide

Please note that by not adhering to the protocol unsatisfactory results regarding yield and quality of the plasmid DNA will occur! If problems arise, check the following points:
1. Solution D5 was reconstituted correctly.
2. Solution D4 was shaken well immediately before use.
3. All solution volumes, temperatures, incubation times and centrifugation conditions were carried out precisely.
4. The solutions are stored at room temperature.

Low yields!
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 0.1 µg/ml (low copy) to 5.0 µg/ml (high copy). Additionally, the size and sequence of specific DNA inserts influences the copy number of a particular plasmid, and thus the yield of plasmid DNA.
2. It is recommended to dry the JETPREP resin (step 7) until the resin is totally white. This is achieved after a few minutes under vacuum. If the drying procedure is too long and the pellet is overdried, the plasmid DNA cannot be recovered quantitatively from the resin.

RNA contamination!
The JETPREP Miniprep Procedure includes two steps to remove the bacterial RNA. The first step is the RNase digestion during cell lysis (after addition of solution D2). Most of the bacterial RNA is degraded during the 5 min of incubation, before solution D3 is added. In a second step, residual mature RNA and RNA degradation products are removed during plasmid binding and washing of the JETPREP resin. Under the given buffer conditions the JETPREP resin is able to distinguish between double-stranded plasmid DNA and single stranded RNA. If residual RNA occurs in the eluted plasmid DNA, washing of the JETPREP resin was probably unsatisfactory. It is necessary to wash the resin by resuspending it in the wash solution D5. Additionally, after centrifugation, the wash solution must be removed quantitatively to avoid residual RNA contaminating the eluted plasmid DNA.

Chromosomal DNA contamination!
Chromosomal bacterial DNA is removed from the preparation by precipitation after the addition of solution D3 and after centrifugation. This is only successful if shearing of the chromosomal DNA after cell lysis is kept minimal. Shearing of the chromosomal DNA occurs when the sample is vortexed after the addition of solution D2 and/or solution D3. 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 plasmid supercoiled form is due to irreversible denatured plasmid DNA. The irreversible denaturation is probable if the cell lysis (step 3 of the protocol) was carried out for longer than the recommended 5 min under the strongly alkaline conditions.

General recommendations!
1. Best results in plasmid isolation can be achieved by the growth of E.coli bacteria in LB (Luria-Bertani) medium. Bacteria grown in very rich media should be diluted, respectively.
2. Avoid having too many beads of the resin in the transferred elute (step 7 of the protocol). If this is the case, recentrifuge and transfer the supernatant to a new tube.