

Product List

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Protocol

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JETstar

The
Novel
Plasmid
Purification
System

Mini
Midi
Maxi



Description

JETSTAR is a new and unique **anion exchange resin** developed by GENOMED. This new resin is supplied in **disposable columns (Mini, Midi and Maxi columns)**, which are used under gravity flow conditions without further instrumentation. The **JETSTAR Plasmid Kits** were designed by combining **JETSTAR Columns** with a modified alkaline/SDS lysis procedure for the preparation of plasmid DNA. GENOMED's product is the ideal tool to achieve **ultrapure plasmid DNA within 45 - 60 min.**

Procedure: The procedure employs a modified alkaline/SDS method to prepare the cleared lysate. After neutralization, the lysate is applied onto a **JETSTAR Column (Mini, Midi or Maxi)** and the plasmid DNA is bound to the **anion exchange resin**. Washing the resin removes RNA and all other impurities. Finally the purified plasmid DNA is eluted from the column and concentrated by an alcohol precipitation.

Plasmid yields: Yields of **up to 20 µg (Mini), 100 µg (Midi) and 500 µg (Maxi) of plasmid DNA** can be expected using the JETSTAR columns. All types and sizes of plasmid DNA can be prepared, but yields depend very much on the plasmid copy numbers (low/medium/high), the type of plasmid, the bacterial strain and the volume of bacterial culture used. The recovery of plasmid DNA is on average between 85% and 90%.

Plasmid purity: The JETSTAR-purified plasmid DNA is of higher quality than 2 x CsCl purified plasmid DNA. Correspondingly, its recommended application range is extremely wide, including **transfection, microinjection, vaccination, gene therapy, fluorescent and radioactive sequencing, amplification, *in vitro* transcription, cloning, etc.**

Culture Volumes and DNA Yields

JETSTAR Kits are preferentially designed to extract and purify high copy plasmid DNA from *E.coli* cultures. Low copy plasmids can be prepared as well, but larger culture volumes have to be used.

GENOMED recommends LB medium to grow *E.coli* cells to prepare plasmid DNA with JETSTAR columns. The JETSTAR system is however compatible also with other growth media, especially those designed for enhanced plasmid yields. The cell density should be approximately 1×10^9 cells per ml medium (1-1.5 A₆₀₀ units/ml).

High Copy Plasmids (3 - 5 µg DNA/ml LB medium)

| A. | Culture Volume | DNA Yield |
|------|----------------|--------------|
| Mini | 1 - 5 ml | 3 - 20 µg |
| Midi | 15 - 25 ml | 45 - 100 µg |
| Maxi | 100 ml | 300 - 500 µg |

Low Copy Plasmids (0.2 - 1 µg DNA/ml LB medium)

| A. | Culture Volume | DNA Yield |
|------|----------------|-------------|
| Mini | 10 - 15 ml | 2 - 15 µg |
| Midi | 25 - 100 ml | 5 - 100 µg |
| Maxi | 250 - 500 ml | 50 - 500 µg |

Protocol for Mini, Midi and Maxi

Dissolve the lyophilized RNase A in solution E1 as recommended.

1. Column Equilibration Columns are equilibrated before the cleared lysate is prepared by applying **solution E4**. The flow of solution starts automatically. Allow the column to empty by gravity flow. **Do not force out** remaining solution.

Solution E4 / Mini: 2 ml
Midi: 10 ml
Maxi: 30 ml

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

3. Cell Resuspending Add **solution E1** to the pellet and resuspend the cells until the suspension is homogeneous.

Solution E1 / Mini: 0.4 ml
Midi: 4.0 ml
Maxi: 10.0 ml

4. Cell Lysis Add **solution E2** and mix gently, but thoroughly **by inverting** until the lysate appears to be homogeneous. **Do not vortex!** Incubate at **room temp.** for **5 min.**

Solution E2 / Mini: 0.4 ml
Midi: 4.0 ml
Maxi: 10.0 ml

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5. Neutralization Add **solution E3** and mix **immediately** by multiple inverting until a homogeneous suspension is obtained. **NO REMAINDERS OF THE VISCOUS MATTER THAT APPEARED AFTER CELL LYSIS (Step 4) MUST BE LEFT! Do not vortex!** Centrifuge the mixture at **room temperature** and **≥12.000 x g** for **10 min.**

Solution E3 / Mini: 0.4 ml
Midi: 4.0 ml
Maxi: 10.0 ml

6. Column Loading Apply the supernatant from **step 5** to the **equilibrated JETSTAR** column. Allow the lysate to run by gravity flow.

7. Column Washing Wash the column with **solution E5 twice (Mini and Midi)** or **once** when **Maxi** is used. Allow the column for each wash to empty by gravity flow.

Solution E5 / Mini: 2 x 2.5 ml
Midi: 2 x 10.0 ml
Maxi: 1 x 60.0 ml

8. Plasmid Elution Elute the DNA with **solution E6**. **Do not force out** remaining solution.

Solution E6 / Mini: 0.9 ml
Midi: 5.0 ml
Maxi: 15.0 ml

9. Plasmid Precipitation Precipitate the DNA with **0.7 volumes of isopropanol (Mini: 0.63 ml, Midi: 3.5 ml or Maxi: 10.5 ml)**. Centrifuge at **4°C** and **≥12.000 x g** for **30 min.** Wash the plasmid DNA with **70% ethanol** and recentrifuge. Air dry the pellet for **10 min**, and redissolve the DNA in a suitable volume of buffer (i.e. 10 mM Tris-Cl, pH 8,0, TE buffer or water).

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Comments on the Protocol

Comments on step 1: Equilibration takes about 10 - 15 min, but it does not influence the speed of the protocol as you can proceed to step 2 during equilibration.

Comments on step 2: Make sure that culture medium back-draining from the tube's wall is removed. Last traces of medium should be removed with a pipette. Residual culture medium will change the balanced conditions of all the following steps of the protocol.

Comments on step 4: To avoid shearing of the genomic DNA, do not vortex the mixture. The following incubation should not take longer than 5 minutes, otherwise plasmid DNA might be denatured irreversibly. - If SDS is precipitated in solution E2 due to low transport or storage temperatures, redissolve it by warming in a water bath at 37°C for a few minutes.

Comments on step 5: When solution E3 is taken out of its bottle, make sure that no liquid sticks to the pipette tip's outer side. A surplus of solution E3 may cause lower plasmid yields. - The centrifugation to pellet potassium dodecyl sulfate (PDS) is performed best when the mixture is homogeneous. Thus, mix the tube gently, but thoroughly by inverting until a HOMOGENEOUS suspension is obtained. **Do not vortex!** - When many samples are prepared in parallel, each sample should be mixed immediately after adding solution E3. - In order to achieve an optimal yield of pure plasmid DNA, it is necessary that after centrifugation the temperature of the sample is at 18° - 25°C. It is important to make sure that the cleared lysate is not at 4°C when loaded onto the JETSTAR column. - After centrifugation save the supernatant with a pipette, and avoid getting too many PDS particles into the transferred supernatant.

Comments on step 6: It is recommended to apply the sample onto the column with a pipette. - When the lysate has run through the column, but drops are still adhering to the column's wall, wash them down with wash solution in step 7.

Comments on step 9: Be careful when drying the DNA pellet under vacuum in a speed-vac or in a vacuum chamber using an oil pump or a water jet filter pump. This may cause overdrying of the DNA pellet so that the DNA cannot be redissolved completely.

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, please check the following points:

- 1. All conditions regarding volumes, temperatures, incubation times and centrifugations were kept precisely.**
- 2. Solutions were stored at recommended temperatures.**
- 3. Columns were equilibrated with solution E4 before use.**
- 4. JETSTAR 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!

- The temperature of the solutions E1-E6 was too low (below room temperature). Especially cold lysate (a chilled centrifuge was used in step 5 of the protocol) and cold washing solution E5 (stored in a refrigerator) will cause decreasing yields.
 - 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 may influence the copy number of a particular plasmid and so the yield of the plasmid DNA.
 - It is very important that the specified volumes of solutions E1-E3 are kept precise. Also, the required ratio of the solutions
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E1, E2 and E3 can be destroyed, if back-draining culture medium is present or too much solution E3 is added for neutralization. A surplus of E3 can be caused by drops sticking to the outside of the pipette tip when solution E3 is taken out of its bottle.

4. The plasmid DNA is overdried and can hardly be redissolved, thus causing low DNA yields. This can be avoided when the DNA pellet is air dried. When vacuum generated by an oil pump is used, the drying period should not exceed 5 min.

Chromosomal DNA contamination!

Chromosomal bacterial DNA is removed from the preparation by precipitation after the addition of solution E3 and by centrifugation. 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 E2 or solution E3. **Avoid vortexing during steps 4 and 5 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 likely to occur if cell lysis (protocol, step 4) is carried out for longer than the recommended 5 min under the strongly alkaline conditions.

Insoluble particles!

In the case that a few particles become visible with the redissolved plasmid DNA (protocol; step 9), the quality of plasmid DNA is not compromised. Nevertheless, the particles can be removed easily by a short centrifugation. Therefore, centrifuge the plasmid DNA solution for 2 min. and transfer the supernatant into a new tube.

RNA contamination!

1. Due to the nature of the anion exchange resin the separation of DNA and RNA is strongly dependent on the salt concentration, the pH-value and the temperature during binding, washing and elution. Therefore avoid changes in conditions and volumes of solutions E1-E6. See **comment 3** of section "**Low yields of plasmid DNA**".

2. When the column procedure has started (lysate has been applied) proceed with column washing and DNA elution without significant interruptions. Long interruptions between the steps of the column procedure may cause contamination of the purified plasmid DNA with small RNA species.

3. When the lysate is applied to the column, residual liquid of the lysate on the column's wall should be avoided. Wash down drops of lysate with the wash solution. Allow the wash solution to run completely through the column before the second round of wash buffer or the elution buffer is applied.

4. The temperature of the sample (after step 5 of the protocol) applied to the column was too high, due to the fact that the sample warmed up during centrifugation.

5. RNase A digestion was insufficient. Check the culture volumes against recommended volumes (see page 2). If solution E1 (containing RNase) is older than 6 months, add new RNase A.

6. Some host strains are extremely rich of RNA. Therefore, it is possible that a slight contamination of the plasmid DNA with residual RNA might occur, even in the presence of RNase. This contamination can be minimized when the protocol is modified by using 10 % more of solution E3 in step 5 of the protocol.

Solutions

All solutions supplied with the kit are ready to use. Only the lyophilized RNase must be added to solution E1 (see note with the RNase tube). The final concentration of RNase is 100 µg per ml E1.

| | |
|---|-----------------------|
| Solution E1 (Cell Resuspending) | Store at RT |
| 50 mM Tris | |
| 10 mM EDTA | |
| HCl ad pH 8.0 | |
| The RNase-containing solution E1 must be stored at 4 °C. | |
| Solution E2 (Cell Lysis) | Store at RT |
| 200 mM NaOH | |
| 1.0 % SDS (w/v) | |
| Solution E3 (Neutralization) | Store at RT |
| 3.1 M potassium acetate | |
| acetic acid ad pH 5.5 | |
| Solution E4 (Column Equilibration) | Store at RT |
| 600 mM NaCl | |
| 100 mM sodium acetate | |
| 0.15 % TritonX-100 | |
| acetic acid ad pH 5.0 | |
| Solution E5 (Column Washing) | Store at RT |
| 800 mM NaCl | |
| 100 mM sodium acetate | |
| acetic acid ad pH 5.0 | |
| Solution E6 (DNA Elution) | Store at RT |
| 1250 mM NaCl | |
| 100 mM Tris | |
| HCl ad pH 8.5 | RT = room temperature |

Notes
