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Plasmid DNA Purification Mini Spin Column Kit

Kit for the purification of plasmid DNA from
E. coli and other bacteria

Version: 22092011

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- Cell Culture Products
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- Molecular Biology Products
- PCR
- Proteins and Enzymes
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Related Products

Mini Spin Column DNA Purification Kit	Contents	Cat. No.
Plasmid DNA Kit Purification of plasmid DNA	50 reactions	S5369.0050
	250 reactions	S5369.0250
GEL Extraction Gel extraction of fragments and plasmids	50 reactions	S5374.0050
	250 reactions	S5374.0250
JustSpin GEL Extraction Gel extraction of fragments and plasmids	50 reactions	S5337.0050
	250 reactions	S5337.0250
PCR Kit Purification of PCR products	50 reactions	S5368.0050
	250 reactions	S5368.0250
PSI Clone High Throughput PCR Kit Purification of PCR products	50 reactions	S5303.0050
	250 reactions	S5303.0250

Coming Soon - Related Products

Mini Spin Column DNA Purification Kit	Contents	Cat. No.
Genomic DNA BAC Gram-positive and gram-negative bacteria	50 reactions	
	250 reactions	
Genomic DNA Plant Plants and soil	50 reactions	
	250 reactions	
Genomic DNA Food Food and feed of plant or animal origin	50 reactions	
	250 reactions	
Genomic DNA Tissue Tissue including mouse tail	50 reactions	
	250 reactions	
Genomic DNA Blood & Cell Cultures Blood and cell culture	50 reactions	
	250 reactions	

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Storage Conditions and Stability

Store RNase A vial at –20 °C until use. RNase A can be stored under this condition for at least 1 year. The kit shows full performance for at least one year, if stored dry at room temperature (15-25 °C). Precipitates in buffers should be dissolved by warming to 37°C. Close bottles immediately after use.

Guarantee for full performance of the kit as specified in this handbook is only valid if storage conditions are followed.

Limited License

The purchase price paid for the **Plasmid DNA Purification Mini Prep Kit** by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended for internal research only by the purchaser. Furthermore, research only use means that the **Plasmid DNA Purification Mini Prep Kit** and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without written approval of the manufacturer.

Separate licenses are available from the manufacturer for the express purpose of non-research use and applications. To inquire about such licenses, or to obtain permission to transfer or use the enclosed material, please contact your local distributor.

Limitations of Product Use

The use of this kit is strictly limited to research purposes. They are not to be applied for any diagnostic, including human, or drug purposes. This also excludes administration to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the regulatory authorities in the country of use. All due care and attention should be exercised in handling of the materials described in this handbook.

Before using a PCR Purification Kit, customers and other users should make their own determination that the product is suitable for intended use. They should ensure that they can use the PCR Purification Kit product safely and legally. This document does not constitute a warranty or assume any liabilities on behalf of the manufacturer except in writing signed by the manufacturer. Unless otherwise agreed in writing, the exclusive remedy for all claims is replacement of the product or refund of the purchase price at manufacturer's option, and in no event shall the manufacturer be liable for special, consequential, incidental, punitive or exemplary damages.

Quality Control

Genaxxon bioscience is dedicated to your success and every batch of this product is tested with an extensive routine procedure to make sure that it meets all your needs. However, it has neither been developed nor tested for a specific application.

We reserve the right to change, alter, or modify our Plasmid DNA Purification Mini Prep Kit to enhance its performance and design.

This product is for research use only.

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Introduction

This **PLASMID DNA Mini Prep Kit** presents remarkable features of timesaving, easy, prompt and low cost plasmid DNA purification from *E. coli* and other bacteria. The kit is useful for quick preparation of high copy number plasmids in quantities (up to 20µg) suitable for all downstream applications including sequencing and transfection.

A special feature of this **PLASMID DNA Mini Prep Kit** is that low copy number plasmids and high molecular size plasmids, including vectors like cosmids and fosmids, can be isolated easily at quantities of up to 7µg for subsequent analytical applications.

Plasmid DNA purified by the **PLASMID DNA Mini Prep kit** is ready for use for a broad panel of downstream applications:

- PCR
- Restriction enzyme digestion
- Labeling
- Ligation
- Transformation
- Transfection
- Sequencing
- In vitro transcription

Name	Amount of starting material	Yield	Approx. time for preparation
PLASMID DNA Mini Prep kit	1 – 6mL of bacterial culture	Up to 20µg	15 min for up to 4 reactions 45 min for 20 reactions

Troubleshooting

Inefficient lysis among other factors can decrease the yield of plasmid DNA. Attention should be paid also to the use of media that can influence the plasmid yield. We recommend LB (Luria-Bertani) medium (5g yeast extract, 10g peptone and 10g NaCl per liter) for growth of *E. coli* and other heterotrophic bacteria.

Observation	Possible cause	Suggestions
Poor or low recovery General note	Low yields may be caused by a number of factors. To find the source of the problem, analyze fractions saved from each step in the procedure on an agarose gel. A small amount of the cleared lysate and the entire flow-through can be precipitated by adding 0.7 vol. isopropanol and centrifuging at max. speed (13,000 rpm or ca. 17,000xg) for 30 min.. The entire wash flow-through can be precipitated by adding 0.1 vol. of 3M sodium acetate, pH5.0 and 0.7 vol. of isopropanol.	
Poor or low recovery	Improper washing Elution buffer incorrectly dispensed Poor elution	Confirm the buffers were diluted with the specified volume of isopropanol and ethanol. Keep bottles tightly capped between uses to prevent evaporation. Add elution buffer to the center of the column membrane to ensure that the buffer completely covers the surface of the membrane. Repeat elution or increase elution volume.
Low A260/280 ratio	Purification is incomplete due to column overloading or inadequate lysis	If the system is overloaded low yields and impure DNA are attributable. Decrease the sample volume as necessary.
Enzymatic reactions using recovered DNA do not proceed	DNA concentration is too low High salt content in the final genomic DNA Residual ethanol from the diluted wash solution	Precipitate the DNA with alcohol, then re-suspend DNA in a smaller volume of elution buffer. Precipitate the DNA using ethanol. Centrifuge the column for 1 minute after the wash steps to remove any residual wash solution.

Appendix

DNA precipitation

1. Add sodium acetate (3M, pH5.2) to the DNA-containing solution to a final concentration of 0.3M and mix well. For high recovery of DNA, glycogen or other commercial precipitation supports can be used.
2. Add 2 volumes of chilled ethanol and mix well.
3. Recover the precipitated DNA by centrifugation (> 16,000xg, 4°C, 15 min.).
4. Carefully decant the supernatant and wash the pellet with 70% ethanol (fill the tube halfway and shortly vortex) by centrifugation (> 16,000xg, 4°C, 10 min.).
5. Carefully decant the supernatant and air-dry the pellet at room temperature.
6. Dissolve the DNA in the desired volume of 10mM Tris/HCl, pH8.0 or deionized, sterile water.

Photometric Determination of DNA Concentration and Quality

Determination of DNA concentration is done by UV reading. Correct measurement is only possible if the DNA is free of RNA and readings are at values between 0.1 and 1 absorption units. DNA preparations should be vortexed shortly and diluted accordingly using 10mM Tris/HCl or water. As a blank, you can use buffer EB diluted at the same factor as the DNA sample:

DNA concentration ($\mu\text{g/ml}$) = $(A_{260\text{nm}} - A_{320\text{nm}}) \times 50$ (DNA extinction coefficient) \times dilution factor.

DNA yield (μg) = DNA concentration \times sample volume (mL)

A standard procedure of measuring DNA quality is the determination of the absorption quotient (Q) of readings at $A_{260\text{nm}}$ and $A_{280\text{nm}}$:

$$Q = (A_{260\text{nm}} - A_{320\text{nm}}) / (A_{280\text{nm}} - A_{320\text{nm}})$$

For a pure DNA preparation, Q lies between 1.7 and 2.0.

Safety Information

It is strongly recommended to wear a lab coat, disposable gloves and protective goggles when working with chemicals. More detailed information is available in the material safety data sheets, which can be requested from the manufacturer.

Caution: Do not add bleach or acidic solutions to the waste of sample preparation.

Risk and safety phrases with relevance:

Buffer 3.0 Contains guanidine hydrochloride, which is harmful and an irritant. See risk and safety phrases R22-36/38, 13-23-26-36/37/39-46.

Guanidine hydrochloride can form highly reactive compounds in combination with bleach. When spilled, clean with suitable detergent and water. Areas affected with spilled infectious agents-containing liquids should be decontaminated with laboratory detergent and water, afterwards with 1% (v/v) sodium hypochlorite.

RNase A:

Ribonuclease A is a sensitizer. See risk and safety phrases R42/43, S23-24-26-36/37.

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Contents of the kits

	4	50	250
Mini spin columns	4	50	250
Microtubes 1.5mL	4	50	250
Receivertubes 1.5mL	4	50	250
Receivertubes 2.0mL	4	50	250
Buffer 1.0 *	1mL	12.5mL	5 x 12.5mL
Buffer 2.0	1mL	12.5mL	5 x 12.5mL
Buffer 3.0	1.4mL	17.5mL	5 x 17.5mL
Buffer 4.0 **	0.56mL add 2.4mL ethanol	7mL add 28mL ethanol	5 x 7mL add 5 x 28mL ethanol
Buffer 5.0	0.8mL	10mL	5 x 10mL
RNase A	0.05mL store at -20°C	0.5mL store at -20°C	2 x 1.25mL store at -20°C
Handbook	1	1	1

Note before getting started:

* Buffer 1 is ready for use after addition of RNase A. For this, add the contents of the RNase A vial to buffer 1 by pipetting, close the bottle and mix by vigorous shaking. Store bottle in the refrigerator (4-12 °C) for up to 4 months. Alternatively, if longer storage of RNase A is desired (for up to 1 year), place RNase A vial in the freezer (-20 °C). Pipette 10 μL of RNase A solution per 250 μL buffer 1 (one reaction).

** Absolute ethanol, p.a. (95-99,8 %).

Additional Material Required

- Microcentrifuge (\geq 13,000 rpm)
- 95-99.8 % ethanol

Protocol

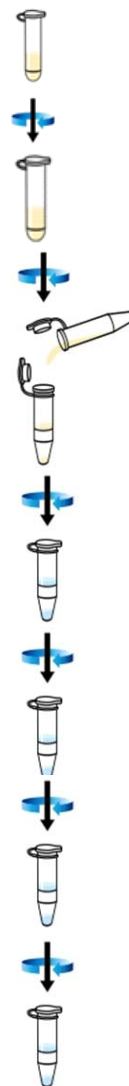
Notes before getting started

- Add RNase A to Buffer 1.0 (see page 4)
- Add ethanol to Buffer 4.0 (see page 4)

Procedure:

1. Transfer 1-4mL of stationary culture (*E. coli*, over night grown) to a 1.5mL micro tube. Centrifuge at $\geq 13,000$ rpm for 30-60 sec. to sediment cells. For volumes greater than 1.5mL, decant supernatant add further culture to the same tube and centrifuge again. Finally, after decanting remove residual liquid by pipetting.
2. Resuspend cell pellet in 250 μ L **Buffer 1.0** (ensure that RNase A has been added to Buffer 1.0) by vigorous vortexing. Pipetting up and down may accelerate resuspension. **Note:** Make sure that small cell pellets or clumps are resuspended completely.
3. Add 250 μ L **Buffer 2.0**, close the tube and mix carefully by inverting 4-6 times. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. **Note:** At this step cell lysis occurs. Do not extend reaction for more than 5 minutes, because otherwise plasmid DNA may denature irreversibly!
4. Add 350 μ L **Buffer 3.0**, close the micro tube and mix carefully by inverting 4-6 times. **Note:** During neutralization denatured chromosomal DNA and proteins are precipitated (the solution should become cloudy).
5. Centrifuge at $\geq 13,000$ rpm for 7 min. During this time, put a mini spin column into a 2.0mL receiver tube.
6. Transfer (decant) the clarified supernatant into the mini spin column and close the tube. Centrifuge at $\geq 13,000$ rpm for 30 sec..
7. Add 700 μ L **Buffer 4.0**. Centrifuge at $\geq 13,000$ rpm for 30 sec..
8. Discard the filtrate (flow-through). Put the mini spin column back into the receiver tube, close lid and dry the filter by centrifugation at $\geq 13,000$ rpm for 30 sec..
9. Place the mini spin column into a supplied 1.5mL receiver tube and add 30-100 μ L **Buffer 5.0** or deionized water directly in the center of the membrane. **Incubate** for 1 min. Finally, centrifuge at $\geq 13,000$ rpm for 1 min to eluate the plasmid DNA.

Note: DNA yield depends on the elution volume, i.e. a higher yield is obtained with a higher elution volume. Alternatively, repeat elution (e.g. 100 μ L) with fresh EB and combine eluates. For highly concentrated plasmid DNA, elute with 30 to 50 μ L **Buffer 5** or deionized water



1-5mL culture

$\geq 13,000$ rpm, 30-60 s

resuspend in 250 μ L **Buffer 1.0+RNaseA**, vortex lysate with 250 μ L **Buffer 2.0**, invert 4-6 times neutralize with 350 μ L **Buffer 3.0**, invert 4-6 times

$\geq 13,000$ rpm, 7 min

transfer the supernatant into mini spin column

$\geq 13,000$ rpm, 30 s

add 700 μ L **Buffer 4.0**

$\geq 13,000$ rpm, 30 s

dry, $\geq 13,000$ rpm, 30 s

add 30-100 μ L, Buffer 5.0 or deionized water, wait 1 min

$\geq 13,000$ rpm, 1 min

eluted plasmid DNA