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Low Copy Plasmid MiniPrep Kit — Product Insert

Product # 17800

Norgen's **Low Copy Plasmid MiniPrep Kit** is designed for the rapid preparation of low copy number plasmid DNA from small batch cultures of *Escherichia coli*. A typical low copy number plasmid only replicates to 2 – 10 copies per cell. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The process does not require ethanol or other alcohols, a significant improvement over other techniques to achieve full compatibility with alcohol sensitive applications. The plasmid DNA is preferentially purified from other cellular components such as genomic DNA and RNA. The purified DNA is fully digestible with all restriction enzymes tested, and is completely compatible with manual or automated sequencing to achieve 95-100% accuracy.

The Low Copy Plasmid MiniPrep Kit contains sufficient materials for 25 preparations. Typical yield from a 3.0 mL culture for a low copy number plasmid is from 0.5 to 1.0 µg. Preparation time for 12-24 samples is approximately 30 minutes. The process does not require ethanol or other alcohols. Purification of plasmids up to 14 kb in size have been verified. The kit has a shelf life of at least 1 year when stored as suggested.

Kit Components

Component	Product # 17800 (25 samples)
Resuspension Buffer	6 mL
Lysis Solution	6 mL
Binding Solution	20 mL
Wash Solution	60 mL
Elution Buffer	6 mL
RNAse A (lyophilized)	8500 units
Micro Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers. The **Resuspension Buffer** should be stored at 4°C upon addition of RNAse A enzyme.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Binding Solution** and **Wash Solution** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes prior to use:

- Ensure that all solutions, except the **Resuspension Buffer,** are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Reconstitute the **RNAse A** in 100 µL of the **Resuspension Buffer**. Once it is fully resuspended, add the entire amount of **RNAse A** to the **Resuspension Buffer**. The label on the bottle has a box that can be checked to indicate that the RNAse A has been added. The solution can be stored for up to 6 months at 4°C.
- Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure.

1. Lysate Preparation

a. Transfer 1.5 mL of bacterial culture to a microcentrifuge tube and centrifuge for 20 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add an additional 1.5 mL of bacterial culture to the tube and centrifuge for 20 seconds. Pour off the supernatant carefully as in step **1a.**

c. Add 150 µL of **Resuspension Buffer** (containing **RNAse A**; see Notes Prior to Use) to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing. Incubate at room temperature for 5 minutes.

d. Add 150 µL of **Lysis Solution** to the cell suspension, cap the tube, and mix the contents by gently inverting the tube several times. Do not vortex as this will shear the genomic DNA. The suspension should become clear and viscous as the cells begin to lyse. Continue mixing until the mixture becomes clear. If necessary, allow the solution to incubate at room temperature provided the total incubation time is no more than 5 minutes. This step is also critical for the denaturation of cellular proteins and genomic DNA.

e. Add 350 µL of **Binding Solution** and immediately mix by inverting the tube several times. The solution will become turbid as insoluble particles from denatured materials start to form.

f. Centrifuge for 10 minutes to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

2. Binding to Column

a. Assemble a spin column with a provided collection tube. Transfer the lysate into the micro spin column, ensuring that none of the white particulates from step **1f** are transferred onto the column. Cap the column, and then centrifuge the unit for 1 minute.

b. After centrifugation, separate the column from its collection tube. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Washing Bound DNA

a. Apply 60 µL of **Wash Solution** to the column, and centrifuge for 2 minutes.

b. Ensure that all the **Wash Solution** has passed through the column and that the column is dry. Spin for an additional minute if necessary

c. Discard the flowthrough and reassemble the column with a provided **Elution tube**.

4. Elution of Clean DNA

a. Add 30-50 µL of **Elution Buffer** to the column and centrifuge for 2 minutes at **2,000 x g**. Centrifuge the elution for an additional 1 minute at **14,000 x g.**

For more concentrated DNA use a 30 µL elution volume. If higher recoveries are required, use a 50 µL elution volume.

Note: If the speed of the centrifuge can not be adjusted, the elution of DNA can also be performed at 14,000 x g for 1 minute. Lower recoveries of DNA may be expected.

b. (Optional): An additional elution can be performed by repeating step **4a**. This elution should be collected into a separate tube to avoid diluting the DNA solution in the first elution.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Plasmid did not propagate	Ensure that the appropriate growth medium, supplements and antibiotics were used for the host cell and plasmid of interest.
	Inoculum cell culture was old	Old bacterial cells are a poor source of plasmid DNA. Bacterial cell inoculumn should be prepared from fresh single colonies, grown in a test-tube overnight and immediately used for inoculum preparation. Prolonged incubation or storage of culture in the fridge almost guarantees poor results.
	Insufficient lysis of cells	The **Lysis Solution** may have formed precipitates. Warm and mix gently before use.
	Cell resuspension was incomplete	Pelleted cells should be completely resuspended in the **Resuspension Buffer**. Do not add **Lysis Solution** until a homogeneous suspension is obtained.
	Proper **Elution Buffer** was not used	The provided **Elution Buffer** has been optimized for high elution recoveries. If water is used, ensure that the pH is between 7 and 8.
DNA does not perform well in downstream applications	DNA was not washed with the provided **Wash Solution**	Traces of salt from the binding step may remain in the sample if the column is not washed with **Wash Solution**. Salt may interfere with downstream applications, and thus must be washed from the column.
	A different **Elution buffer** was used	If a different **Elution buffer** other than the one provided in the kit was used, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

Related Products	Product #
Norgen Plasmid MaxiPrep Kit	15300
BAC DNA MiniPrep Kit	18000
Norgen Plasmid MiniPrep Kit	13300
PCRSizer 100bp DNA Ladder	11300
HighRanger 1kb DNA Ladder	11900

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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