NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

# Plasmid MiniPrep Kit

# Product Insert

**Product # 13300 & 46400**

Norgen's Plasmid MiniPrep Kit is designed for the rapid preparation of plasmid DNA from small batch cultures of *Escherichia coli*. The plasmid DNA is preferentially purified from other cellular components such as genomic DNA and RNA. The Plasmid MiniPrep Kit is able to purify plasmids up to 13,000 bp in size, and the typical purification yield is up to 20 μg from 1.5 mL of bacterial culture. 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.

**Norgen's Purification Technology**

Purification is based on spin column chromatography. The process for the isolation of plasmid DNA involves first pelleting 1.5 mL of an overnight culture of *E. coli* harbouring the plasmid of interest using centrifugation (please see the flow chart on page 4). The pellet is then resuspended in the provided Resuspension Solution AZ, which contains RNase A. Lysis Buffer N is then added to the sample in order to assist in the lysis of the bacterial cells. Next, Buffer TN is added to the sample which will neutralize the sample and cause precipitation of the proteins and genomic DNA that is present. The resulting suspension is spun down, and the clarified lysate containing the plasmid DNA is then applied to a provided column through centrifugation. Norgen's column binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the column while most of the RNA, proteins and other contaminants will either flowthrough or be retained on top. The bound DNA is then washed twice using the provided Wash Solution E in order to remove any remaining impurities, and the purified PCR product is eluted with the Elution Buffer K.

## Specifications

| Kit Specifications | |
|---|---|
| Column Binding Capacity | 25 μg |
| Size of Plasmids Purified | Up to 13,000 bp |
| Average Yield from 1.5 mL of Culture | Up to 20 μg |
| Time to Complete 10 Purifications | 30 minutes |

## Advantages

- Fast and easy processing using a rapid spin-column format
- High column binding capacity – up to 25 μg
- High yield of plasmid DNA – up to 20 μg from 1.5 mL of culture

## 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 RNase should be stored at -20°C upon arrival. The **Resuspension Solution AZ** should be stored at 4°C upon addition of RNase enzyme.

**Kit Components**

| Component | Product # 13300 (50 samples) | Product # 46400 (250 samples) |
|---|---|---|
| Resuspension Solution AZ | 12 mL | 60 mL |
| Lysis Buffer N | 40 mL | 80 mL |
| Buffer TN | 20 mL | 130 mL |
| Wash Solution E | 12 mL | 2 x 18 mL |
| Elution Buffer K | 8 mL | 30 mL |
| RNase A | 1 vial | 1 vial |
| Spin Columns | 50 | 250 |
| Collection tubes | 50 | 250 |
| Elution tubes (1.7 mL) | 50 | 250 |
| Product Insert | 1 | 1 |

**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 **Buffer TN** contains 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
- 96 – 100% ethanol

# Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. Please check your microcentrifuge specifications to ensure proper speed. 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.

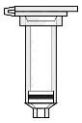
## Flow Chart

Procedure for the Rapid Preparation of Plasmid DNA

Pellet overnight bacterial culture containing plasmid of interest

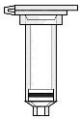
Resuspend in Resuspension Solution A,
Lyse using Lysis Buffer N,
Add Buffer TN to Neutralize

**SPIN**



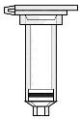
Bind Clarified
Lysate to Column

**SPIN**



Wash with
Wash Solution E

**SPIN**



Elute with
Elution Buffer K

**SPIN**

**Pure Plasmid DNA**

**Notes prior to use:**

- All centrifugation steps should be carried out at 14,000 x g (~14,000 RPM).
- Ensure that all solutions, except the **Resuspension Solution AZ,** 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.
- Add the entire amount of **RNase** to the **Resuspension Solution AZ**. The label on the bottle has a box that can be checked to indicate that the RNase has been added. The solution can be stored for up to 6 months at 4°C.
- **Product # 13300 (50 Prep Kit)**: Prepare a working concentration of **Wash Solution E** by adding 48 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution E**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- **Product # 46400 (250 Prep Kit):** Prepare a working concentration of **Wash Solution E** by adding 72 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution E**. This will give a final volume of 90 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Bacterial cultures grown overnight at 37$^{o}$C in LB medium are optimal for this procedure.
- For purification of low copy number plasmid DNA (2 – 10 copies per cell), please refer to the "**Notes**" within the procedure in Step1a and Step 4b.

**1. Lysate Preparation**

**a.** Transfer 1.5 mL of bacterial culture to a microcentrifuge tube and centrifuge for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

**Note:** For purification of low copy number plasmid DNA (2 – 10 copies per cell), add an additional 1.5 mL of the bacterial culture to the cell pellet from Step **1a** and centrifuge for 30 seconds. Pour off the supernatant carefully (as in Step 1a).

**b.** Add 200 µL of **Resuspension Solution AZ** (containing **RNase**; 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.

**c.** Add 250 µL of **Lysis Buffer N** 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.

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

**e.** 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 one of the provided collection tubes, and transfer the lysate into the spin column. Ensure that none of the white particulates from step **1e** 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 600 μL of **Wash Solution E** to the column, and centrifuge for 1 minute.
   - **b.** Discard the flowthrough. Reassemble the spin column with its collection tube.
   - **c.** Spin the column for 2 minutes in order to thoroughly dry the column. Discard the collection tube.

4. **Elution of Clean DNA**
   - **a.** Assemble the spin column (with DNA bound to the column) with a fresh 1.7 mL **Elution Tube** included with the kit.
   - **b.** Add 50 μL of **Elution Buffer K** to the center of the column. It is important to place the **Elution Buffer K** directly over the column's membrane and not on the side of the column, as this will decrease the DNA recovery. Let stand at room temperature for 1 minute then centrifuge the column for 2 minute**.**

     **Note:** For elution of low copy number plasmid DNA (2 – 10 copies per cell), add 30 μL of **Elution Buffer K** to the column. Let stand for 1 minute at room temperature and then centrifuge for 2 minutes. For maximum concentration of the eluted DNA, transfer the eluate back onto the same column and repeat the 1 minute incubation at room temperature, followed by centrifugation for 2 minutes.
   - **c.** (Optional): An additional elution can be performed if desired by repeating step **4b**. 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 inoculum 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 Buffer N** may have formed precipitates. Warm and mix gently before use. |
| | Cell resuspension was incomplete | Pelleted cells should be completely resuspended in the **Resuspension Solution AZ**. Do not add **Lysis Buffer N** until a homogeneous suspension is obtained. |

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Poor DNA Recovery | **Elution Buffer K** was not placed directly over the column's membrane | It is important that the **Elution Buffer K** be placed directly over the column's membrane to ensure uniform passing of the buffer through the column. Do not pipette the **Elution Buffer K** onto the side of the column. |
| | Proper Elution Buffer was not used | The provided **Elution Buffer K** 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 E** | Traces of salt from the binding step may remain in the sample if the column is not washed with **Wash Solution E**. 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 K** 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 # |
|---|---|
| Plasmid MaxiPrep Kit | 46500, 46600 |
| BAC DNA MiniPrep Kit | 18000 |
| 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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362