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Plasmid MiniPrep 96-Well Kit (Magnetic Bead System) Product Insert

Product # 63000

Norgen's Plasmid MiniPrep Kit 96-Well (Magnetic Bead System) is designed for the rapid high-throughput preparation of plasmid DNA from small batch cultures of *Escherichia coli*. Norgen's magnetic beads bind plasmid DNA under optimized salt concentrations and releases the bound plasmid DNA under low salt and slightly alkali conditions. The plasmid DNA is preferentially purified from other cellular components such as genomic DNA and RNA. The purified plasmids are fully digestible with all restriction enzymes tested, and are completely compatible with real-time PCR and NGS. Norgen's Plasmid MiniPrep 96-Well Kit (Magnetic Bead System) can also be integrated with a robotic automation system.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that bind DNA under optimized binding conditions. 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 3). 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 lysate is then spun in order to remove any debris, and the clean lystate is then transferred into a well of the 96-well plate. Magnetic Bead Suspension is then added to the clean supernatant, and the resulting solution is placed on the magnetic separation rack. Only the plasmid DNA will bind to the magnetic beads, while most of the proteins will be removed in the supernatant. The bound plasmid DNA is then washed with 70% ethanol in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer K. The purified DNA can be used in a number of downstream applications.

Specifications

Kit Specifications	
Size of Plasmids Purified	Up to 13,000 bp
Average Yield from 1.5 mL of Culture	Up to 20 μg
Time to Complete 96 Purifications	45 minutes

Advantages

- Fast, reproducible and easy processing using a magnetic bead system
- Isolate high quality plasmid DNA
- Recovered plasmid DNA is compatible with various downstream applications
- High throughput and compatible with an automation robotic system

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 2 year in their unopened containers. The RNase should be stored at -20°C upon arrival. The **Resuspension Solution A** should be stored at 4°C upon addition of RNase enzyme.

Kit Components

Component	Product #63000 (192 samples)
Resuspension Solution AZ	2 x 20 mL
Lysis Buffer N	2 x 40 mL
Buffer TN	1 x 55 mL 1 x 20 mL
Elution Buffer K	2 x 8 mL
RNase A	1 vial
Magnetic Bead Suspension	4 x 1.1 mL
96-Well Plate	2
96-Well Elution Plate	2
Adhesive Tape	2
Product Insert	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
- Magnetic Bead 96-Well Separation Plate
- Multi-channel micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- 65°C incubator
- Nuclease-free water

Procedure

Notes prior to use:
- 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 A 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.
- Bacterial cultures grown overnight at 37oC in LB medium are optimal for this procedure. Preheat an incubator to 65°C.

Flow Chart

Procedure for the Rapid Preparation of Plasmid DNA

Pellet overnight bacterial culture containing plasmid of interest

SPIN

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

SPIN

Transfer supernatant. Add Magnetic Bead Suspension. Mix. Incubate for 5 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Discard supernatant. Add 70% ethanol, mix and incubate for 1 minute.

Repeat ethanol wash step.
Incubate open tube at 65˚C for 5 minutes.

Add Elution Buffer K, mix and Incubate at 65˚C for 10 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Carefully transfer supernatant to Elution Plate.

Pure Plasmid DNA

1. **Lysate Preparation**
   - **a.** Transfer 1.5 mL of bacterial culture to a microcentrifuge tube and centrifuge at 20,000 x g (14,000 rpm) for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.
   - **b.** Add 200 µL of **Resuspension Solution AZ** (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.
   - **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 at 20,000 x g (~14,000 rpm) for 10 minutes to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.
   - **f.** Transfer the lysate into a 96-Well Plate.
   - **g.** Add 20 µL of **Magnetic Bead Suspension** (vortex prior to use) to the mixture above.
   - **h.** Incubate at room temperature for 5 minutes. Gently shake or vortex the 96-Well plate.
   - **i.** Proceed to Section 2: Plasmid DNA Isolation.

2. **Plasmid DNA isolation**
   - **a.** Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute.
   - **b.** Aspirate and discard supernatant without touching the magnetic beads.
   - **c.** Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of freshly prepared **70% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
   - **d.** Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
   - **e.** Aspirate and discard supernatant without touching the magnetic beads.
   - **f.** Repeat Steps **2c – 2e** for a second wash step.

     Note: Remove as much of the 70% ethanol in the sample plate as possible by pipetting.

   - **g.** Incubate the 96-Well Plate without the magnetic plate at 65˚C for 5 minutes to dry the magnetic beads.
   - **h.** Add 50 µL of **Elution Buffer K**. Mix by vortexing or by pipette and incubate at 65˚C for 10 minutes.
   - **i.** Briefly vortex or mix by pipette and place 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
   - **j.** Carefully transfer the elution to a 96-Well Elution Plate (provided) without touching the magnetic beads. The provided adhesive tape can be used for storage of the DNA. The purified plasmid DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidently pipetted up with the supernatant.	The pipette tip was placed too close to the magnetic beads while pipetting	Return the magnetic beads and the supernatant back into the sample well. Mix well, and place the plate back onto the magnetic separation plate for the specified time. Carefully remove the supernatant without touching the magnetic beads.
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 A**. Do not add **Lysis Buffer N** until a homogeneous suspension is obtained.
	Proper **Elution Buffer K** 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 70% Ethanol (freshly prepared)	Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with **70% Ethanol (freshly prepared)**. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads.
	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 #
Plasmid MiniPrep Kit (Magnetic Bead System)	60300
Plasmid MiniPrep Kit	13300, 46400, 25900
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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