G-Biosciences, St Louis, MO, USA ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

GET™ Plasmid Maxi Prep

For High Yield, Quality Plasmid DNA Extraction

INTRODUCTION

The *GET*™ Plasmid Maxi Prep isolates super-coiled plasmid DNA from 100-500ml *E. coli* cultures. The extraction method is based on alkaline lysis of bacteria and uses our high affinity *GET*™ Plasmid Maxi Columns to bind and purify plasmid DNA from the bacteria. The extracted plasmid is ready for further DNA manipulations, such as restriction enzyme digestion, ligation, transformations, transfections, sequencing and PCR. The plasmid yields are typically up to 100-500µg/prep.

	Cat # 786-363	**Cat # 786-364**
ITEM(S) SUPPLIED	**10 Preps**	**20 Preps**
Cell Suspension Solution	100ml	2 x 100ml
LongLife™ RNase	0.5ml	2 x 0.5ml
Lysis Buffer	250ml	2 x 250ml
Neutralizing Buffer	200ml	2 x 200ml
DNA Wash*	50ml	2 x 50ml
TE Buffer	20ml	2 x 20ml
GET™ Plasmid Maxi Columns	10	20

ITEMS NEEDED BUT NOT SUPPLIED

- Molecular grade Ethanol
- High Speed Centrifuge and microcentrifuge
- 50ml conical tubes, microcentrifuge tubes

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store all reagents at room temperature, except *LongLife*™ RNase, which is to be stored at -20°C. Kits components are stable for 1 year. If a precipitate forms in any buffer, dissolve by warming it at ~50°C.

PREPARATION BEFORE USE

I. Add the entire contents of 1 vial of *LongLife*™ RNase to each Cell Suspension Solution bottle. After addition, store Cell Suspension Solution at 4°C. This is stable for 6 months.
II. To each bottle of DNA Wash, add 200ml molecular grade ethanol. Chill the DNA Wash before use, preferably at -20°C. An ice bucket can be used as an alternative.
III. Warm the TE Buffer at 55-60°C before use.
IV. Chill the Neutralizing Buffer in an ice bath prior to use.

PROTOCOL

1. Harvest the bacterial cells from 100-500ml overnight grown culture by centrifugation at 5,000xg for 10 minutes. Discard the supernatant.

2. Add 10ml ice-cold Cell Suspension Solution to the bacterial pellet. Vortex until a homogenous bacterial cell suspension is achieved. Transfer the cell suspension to a 50ml centrifuge tube.

3. Add 20ml Lysis Buffer and gently invert the tube 15-20 times to mix. DO NOT VORTEX. Incubate at room temperature (~20-25°C) for 5 minutes.

4. Add 20ml ice-cold Neutralizing Buffer. Mix by inversion 15-20 times. DO NOT VORTEX. Incubate on ice for 15 minutes.

5. Transfer the mixture directly to a high speed centrifuge tube and centrifuge mixture for 20 minutes at 20,000x g at 4°C. If any white precipitate remains in the supernatant centrifuge it again for a further 10 minutes then transfer the supernatant to a clean tube.

6. Transfer half of the supernatant to the *GET*™ Plasmid Maxi Column and centrifuge the column at 5000g for 5 minutes at room temperature. Remove the column from the collection tube and discard the flow through liquid. Insert the column back into the collection tube.

7. Repeat step 6 for the remaining supernatant.

8. Add 20ml ice-cold DNA Wash to the column unit and centrifuge at 5,000xg for 5 minutes at room temperature. Remove the column from the collection tube and discard the flow through liquid. Insert the column back into the collection tube.

9. Centrifuge the column at 5,000g for 10 minutes to remove residual wash buffer.

10. Remove the cap and let stand at room temperature for 10 minutes to dry.

11. Transfer the DNA binding column into a clean 50ml tube and add 1ml of prewarmed (50-60°C) TE Buffer or sterile water to the center of DNA binding column and stand at room temperature for 10-15 minutes. Elute the plasmid DNA by centrifuge the unit for 5 minutes at 5,000xg at room temperature.

12. *DNA Precipitation:* Add 0.7 volumes of isopropanol to DNA elution, invert tube to mix. Incubate at room temperature for 5 minutes and then centrifuge immediately at 14,0000g for 10 minutes at 4°C. Carefully remove the supernatant without disturbing the DNA pellet.

13. Wash the DNA pellet with 1ml 70% ethanol and centrifuge at 14,000rpm for 10 minutes. Carefully remove the supernatant without disturbing the pellet.

14. Let the DNA pellet air dry for 15-20 minutes at room temperature, and then dissolve it in appropriate volume of TE Buffer (0.5-1.0ml).

APPLICATION NOTES

The ***GET*™ Plasmid Maxi Prep** is designed to isolate plasmid DNA from *E. coli*. Check literature reference to determine maximal growth conditions, antibiotics to use for each bacterial strain and plasmid combination. It should be noted that plasmid DNA should be harvested while the bacteria numbers are rapidly expanding; not after populations have reached a plateau or declined. In general, OD readings at A_{600} of 1.5-2.0 will provide maximal yields of high quality plasmid DNA.

.

RELATED PRODUCTS

1. ***Nucleic dotMETRIC*™** *(Cat # 786-61) – Allows DNA, RNA and oligonucleotide concentrations to be measured using as little as 1 µl of sample. Measurements take 2 minutes and are perfect for geneEXIT™ or any other use requiring a minimal waste of sample.*
2. ***PinkCLEANUP*™** *(Cat # 786-87 & 88) – DNA Cleanup Kit for removing excess salts, enzymes, unincorporated nucleotides, and primer-dimers from DNA preparations. Ideal applications include PCR clean-up and restriction enzyme removal from plasmid DNA prior to in-vitro transcription.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*