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A Geno Technology, Inc. (USA) brand name

GET™ Plasmid Mini Prep

For High Yield & Quality Plasmid DNA Extraction

(Cat. # 786-361, 786-362)

INTRODUCTION

GET™ Plasmid Mini Prep kit isolates high quality plasmid DNA from 1-5ml E. coli cultures. The kit utilizes an enhanced DNA binding column to produce high yields of plasmid. This quick and easy protocol eliminates toxic phenol/chloroform extractions or lengthy ethanol precipitations. On completion of the protocol, the plasmid DNA is ready for restriction enzyme digestion, sequencing, subcloning and in vitro transcription. The plasmid yields are typically up to 20µg/prep.

ITEM(S) SUPPLIED

Description	Cat # 786-361 50 Preps	Cat # 786-362 100 Preps
Cell Suspension Solution	15ml	1 x 30ml
Longlife™ RNase	0.5ml	0.5ml
Lysis Buffer	20ml	2 x 20ml
Neutralizing Buffer	20ml	2 x 20ml
DNA Wash*	20ml*	2 x 20ml*
TE Buffer	10ml	10ml
GET™ Plasmid Mini Columns with collection tubes	50	2 x 50

*See Preparation before Use

ADDITIONAL ITEMS REQUIRED

- High speed microcentrifuge
- Ethanol 95%, Molecular biology grade
- 1.5ml Centrifuge tubes

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store all reagents at room temperature, except the LongLife™ RNase, which is to be stored at -20°C. Kits are stable for 1 year.

NOTE: *If a precipitate forms in the buffers due to inappropriate storage, warm the buffers to dissolve the precipitate.*

PREPARATION BEFORE USE

a. Add 50µl LongLife™ RNase for every 10ml Cell Suspension Solution directly to the Cell Suspension Solution bottle. After addition, store Cell Suspension Solution at 4°C. This is stable for 6 months.
b. To each bottle of DNA Wash, add 80ml molecular grade ethanol. Chill the DNA Wash to -20°C before use.
c. Chill the Neutralizing Buffer on ice prior to use, DO NOT STORE AT 4°C.
d. Warm the TE Buffer to 55-60°C before use.

PROTOCOL

1. Harvest the bacterial cells from 1-5ml overnight culture by centrifugation at >7,000x g for 3-5 minutes. Discard the supernatant.
2. Ensure that the LongLife™ RNase was added into the Cell Suspension Solution. Add 250µl Cell Suspension Solution to the bacterial pellet and re-suspend.
3. Add 250µl Lysis Buffer. Mix gently by inverting the tube until the lysate is clear, do not vortex. To ensure complete RNA digestion, incubate the tube for 2-5 minutes at room temperature. Do not exceed a 5 minute incubation and do not vortex.
4. Add 350µl chilled Neutralizing Buffer and gently invert 8-10 times to mix. Lysate should contain a thick white precipitate.
5. Centrifuge for 15 minutes at 15,000x g.
6. Ensure the GET™ Plasmid Mini column is in a collection tube and apply the supernatant on to the column. Centrifuge for 30-60 seconds at maximum speed and discard the flow through.
7. Wash the column by adding 500µl DNA Wash and centrifuge for 30-60 seconds at maximum speed. Discard the flow through. Repeat this step once.
8. Perform a final spin of the column for 60 seconds to remove any residual DNA Wash from the sides of the column.
   NOTE: *We recommend placing the columns at 37-55°C for 10-30 minutes to ensure all residual alcohol is removed. This helps eliminate issues with the residual alcohol, including samples leaving agarose gel wells during loading.*
9. Place the column in a clean 1.5ml tube. Elute the plasmid DNA from the column by adding 50µl pre-warmed TE Buffer directly to the column membrane. Incubate for 1-2 minutes at room temperature and then centrifuge for 30-60 seconds. The eluted plasmid is ready for restriction digestion, sequencing and other downstream applications.

APPLICATION NOTES:

Consult reference information to determine optimal growth conditions, antibiotic, etc. for each bacterial strain and plasmid combination. The plasmid DNA should be harvested while bacteria are rapidly expanding and not after populations plateau or decline. Bacterial overgrowth can hamper plasmid yield. In general, OD A_{600} readings of 1.5-2.0 will provide maximal yields of high quality plasmid DNA.

RELATED PRODUCTS

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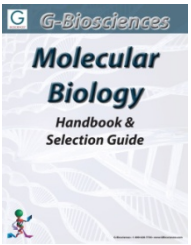

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