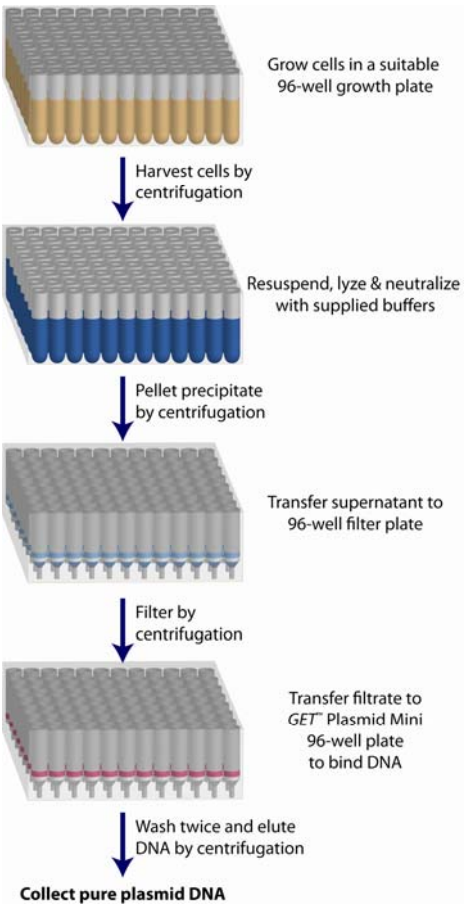
GET™ Plasmid DNA 96-Well

For High Yield and Quality Plasmid DNA Extraction

INTRODUCTION

GET™ Plasmid DNA 96-Well kit is designed for the high throughput preparation of plasmid DNA from 96 x 1-5ml *E. coli* cultures. The kit utilizes a 96 well format enhanced DNA binding matrix 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	Cat # 786-648
	4 x 96 well
Cell Suspension Solution	100ml
Longlife™ RNase	0.5ml
Lysis Buffer	200ml
Neutralizing Buffer	200ml
DNA Wash*	4 x 20ml*
TE Buffer	60ml
GET™ Plasmid Mini 96-well plate	4
96-well filter plate	4
96-well collection plate	8

*See Preparation before Use

ITEMS NEEDED BUT NOT SUPPLIED

- Flat bottom blocks (For growing and lysing bacteria; 96 x 1.3ml bacterial cultures)
- Centrifuge capable of spinning two 96-well blocks stacked (Stacked size: 12.5 x 8.25 x 6cm) at 2,500g
- Ethanol 95%, Molecular biology grade
- Multichannel pipettors (For 50-1,000µl)

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

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 80ml molecular grade ethanol. Chill the DNA Wash to -20°C before use.
III. Chill the Neutralizing Buffer in Ice Bath prior to use, DO NOT STORE AT 4°C.
IV. Warm the TE Buffer to 55-60°C before use.

PROTOCOL

1. Pick a single colony from a freshly streaked plate and inoculate 1-5ml LB media containing appropriate antibiotics. Grow at 37°C for 12-16 hours. Do not incubate longer than 16 hours as cell lysis will occur reducing the plasmid yield. If using a 96-well flat bottom block, add a single colony to each well containing 1ml LB Broth with the appropriate antibiotic. Seal the wells with a plastic lid or adhesive tape. Use a porous tape or punch 2-3 holes above each well with a pin or needle.

2. Harvest the bacterial cells from 1-5ml overnight culture by centrifugation at >7,000x *g* for 3-5 minutes. Discard the supernatant. Use a centrifuge and microtiter plate adaptor for centrifugation of the 96-well flat bottom block. Centrifuge the 96-well flat bottom block at 2,500xg for 5 minutes. Ensure the wells are properly sealed.

3. *Ensure that the LongLife™ RNase was added into the Cell Suspension Solution.* Add 250µl Cell Suspension Solution to the bacterial pellet and re-suspend. Seal the plate with adhesive tape and vortex vigorously until bacterial pellets are resuspended. If cultures were not performed in the 96-well flat bottom block, transfer the bacterial suspension to a 96-well flat bottom block.

4. Add 250µl Lysis Buffer. Dry the top of the 96-well flat bottom block, seal with adhesive tape and mix gently by inverting the tube until the lysate is clear, do not vortex. To ensure complete RNA digestion, incubate the plate for 5 minutes at room temperature. *Do not exceed a 5 minute incubation and do not vortex.*

5. Add 350µl chilled Neutralizing Buffer and dry the top of the 96-well flat bottom block, seal with adhesive tape and mix gently by inverting 8-10 times. Lysate should contain a thick white precipitate.

6. Centrifuge for 5 minutes at 2,500x *g*.

7. Place the 96-well filter plate on top of a 96-well collection plate and transfer the supernatant from step 6 to each well of the filter plate.

8. Centrifuge the stacked plates for 10 minutes at 3,000xg. Discard the 96-well filter plate.

9. Place the *GET*™ Plasmid Mini 96-well plate onto a collection plate and transfer the filtered lysates into the *GET*™ Plasmid Mini 96-well plate.

10. Place the *GET*™ Plasmid Mini 96-well plate on to the collection plate used for the filtered lysates and centrifuge at 2,500xg for 5 minutes.

11. Discard the flow-through from the collection plate and return the *GET*™ Plasmid Mini 96-well plate to the collection plate.

12. Add 500µl DNA Wash to each well and centrifuge the stacked plates at 2,500xg for 5 minutes , discard the flow-through. Repeat the wash step once.

13. Place the *GET*™ Plasmid Mini 96-well plate back on to the collection plate and centrifuge for a further 5 minutes at 2,500xg to remove residual ethanol.

14. Transfer the *GET*™ Plasmid Mini 96-well plate on to a clean collection plate and transfer 50µl warmed TE buffer to each of the wells.

15. Incubate at room temperature for 1-2minutes, then centrifuge the stacked plates for 5 minutes at 2,500xg. The eluted plasmid DNA is now ready for use.
    NOTE: The majority of the DNA will elute at this point, however you may repeat the elution with a further 50µl TE buffer to achieve maximum recovery.

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.

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