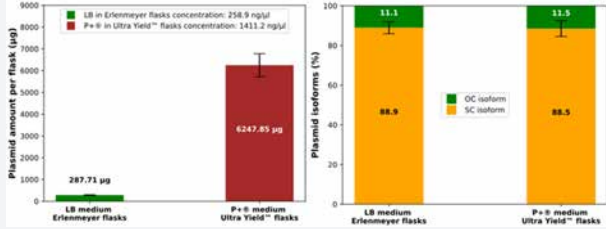


Improved DNA Protocol for *E. coli* with Plasmid+® Media

Show 21X increase over LB while maintaining a high percentage of 85% supercoiled DNA for optimal transfection efficiency



Plasmid+® liquid media is an enriched media specifically designed for plasmid DNA production. Plasmid+® supports much higher cell densities and plasmid yields than LB media. Optimal shake flask yields are achieved using Ultra Yield® Flasks, which facilitates maximum culture aeration. Plasmid+® media may also be used in a bioreactor with continuous aeration and agitation.

Plasmid+® Protocol

1. Use a 1:5 ratio of media for best growth with Ultra Yield® Flasks
2. Add 1:5000 Sigma-Aldrich® Antifoam 204 OR J.T.Baker® Antifoam C Medical Emulsion, Biotech Reagent
3. Starting speed 300-350 RPM in 1-inch/25.4mm orbit for flasks
4. Grow for 22-24 hours (*more time is best*)

Storage

Store PLASMID+® liquid enriched media at room temperature for 24 months from date of manufacture.

Bacteria Strains

E. coli DH5α is the preferred host strain for use with Plasmid+® media. *E. coli* XL1-Blue also produces high quality plasmid DNA and may improve plasmid DNA yields with plasmids smaller than 3kb.

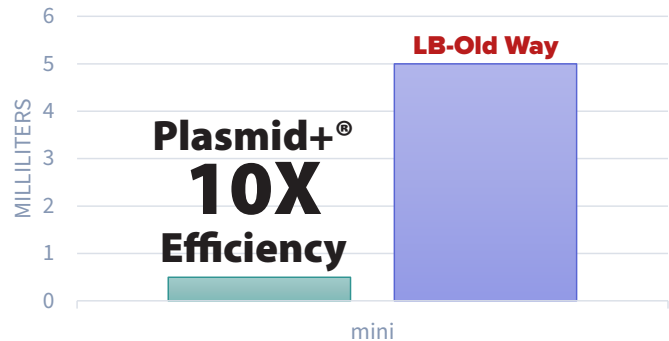
Maximum Column Loading

We recommend resuspending the cell pellet using 10mL of P1 buffer per gram of cell pellet. If preferred, using a volume of P1 buffer equivalent to half of the Plasmid+® culture volume is acceptable, ensure the P2 lysis buffer volumes are appropriate for higher cell density.

10X Increase In Efficiency When Using Plasmid+® vs LB Broth

Plasmid+® reduces the amount of culture required for purification of DNA by 10X and is scalable from mini to giga preparations.

Estimated Culture Volume For Minipreps



Culture Volume Needed

Vented lids for flasks give the best growth over AirOtop® Seals.

| Vessel | Culture volume needed | Yield | Speed/Orbit |
|--------------------|-----------------------|-------|----------------------------|
| 96-Well Plate | 0.5mL – Mini | 20µg | 900RPM 0.2" (3mm) |
| 24-Well Plate | 4mL – Midi | 100µg | 300-350RPM 0.5" (12.5mm) |
| 125mL Ultra Yield® | 30mL – Maxi | 500µg | 300-350RPM 1" (25.4mm) |
| 250mL Ultra Yield® | 75mL – Mega | 2.5mg | 300-350RPM 1" (25.4mm) |
| 2.5L Ultra Yield® | 500mL – Giga | 10mg+ | 300-350RPM 1" (25.4mm) |

Troubleshooting

| | |
|--------------------------------|---|
| Low Protein Yield | <ul style="list-style-type: none"> • Check that the proper antibiotic and concentration is used • Insure proper culture aeration. Use the recommended media volumes in Ultra Yield® Flasks with shaking at 350RPM • Increase the growth time (for up to 48 hours) • Use a starter culture for final culture volumes > 50mL • Protein may be toxic, try growth at 16°C. Growth time may need to be increased at 16°C |
| Low Recovery From Purification | <ul style="list-style-type: none"> • Make sure resuspension of cell pellet is complete • Use enough resin for higher quantity yields |

For the Plasmid+® Datasheet see <https://htslabs.com/datasheet/plasmid-faqsheet>