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Plasmid+® Technical Data Sheet

Product Description	Part #	Sterility
Plasmid+® Media, Sterile, 1L | CS6	446300	Sterile

Introduction

As a component of the Thomson Ultra Yield® system, Plasmid+® media plays a significant role in helping to generate high microbial titers resulting in higher plasmid yields.

Specifications

Size	1L
Final Product	Liquid Medium
Preparation Method	: Ready to Use
Target Organism	*E.coli*
Sterility	Sterile

FAQ's

What is Plasmid+®?

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® Flask and vented cap (Thomson Instrument Company), which facilitates maximum culture aeration. Plasmid+® media may also be used in a bioreactor with continuous aeration and agitation.

How do I store Plasmid+®?

Store Plasmid+® liquid media at room temperature for up to 24 months after manufacture date.

What bacterial strains can be used with Plasmid+®?

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. Other strains can be used, but for DNA production the ones above are the best available.

Do I need to use a seed culture to inoculate?

A seed culture is recommended for culture volumes larger than 50mL. Cultures less than 50mL may be inoculated directly from a glycerol stock or plate. To prepare a seed culture, use a glycerol stock or plate to inoculate 1/100th of the final culture volume of LB broth + appropriate antibiotic (e.g. 100 µg/mL ampicillin; 50 µg/mL kanamycin) and grow to saturation with shaking at 37°C.

How do I use Plasmid+® with the Ultra Yield® Flasks?

Using aseptic technique, add Plasmid+® media and appropriate antibiotic (e.g. 100µg/mL ampicillin; 50µg/mL kanamycin) to one or more Ultra Yield® Flasks; see Table 1 on pg. 2 for recommended culture volumes. Inoculate the media, place an AirOtop® seal on the flask, and grow at 37°C with shaking at 350 rpm for 22 hours.

Do I need to adjust my purification protocol? YES!

Plasmid DNA may be purified from Plasmid+® cultures by the common methods (e.g. Qiagen® Mega kits or Qiagen® Giga kits, etc.). Mega kits we have found starting with 5mgs works well at the high range. Giga kits we have found starting with 20mgs works well at the high range. However, because Plasmid+® media typically yields 5-10 times increased yields when compared to LB media, the increased cell mass and plasmid DNA content must be taken into consideration to ensure efficient lysis and to avoid overloading purification columns. When using plasmid purification kits, the culture volume per purification should be decreased by a factor of 5 with respect to the recommended LB culture volume.

Have you adjusted your protocol and speeds for Plasmid+® enriched media?

Higher RPMs are required while using Plasmid+® enriched media for achieving higher plasmid production.

How do I avoid overloading the purification columns, so I don't lose my DNA?

Plasmid+® creates an enriched DNA cell paste, it is recommended that you resuspend the cell pellet using 10 mL 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.

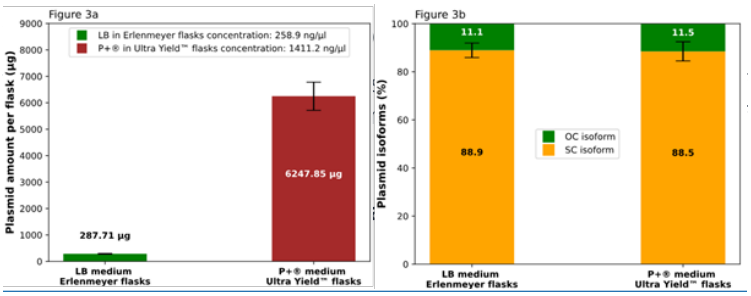

Figure 3: 3a: Average total amount of purified plasmid (in µg) calculated per shake flasks used. Standard cultivation in 250 ml Erlenmeyer flask with a total filling capacity of 10% in green (left), Ultra Yield® flask with 250 ml flask with total filling capacity of 40% in brown (right). Flasks were cultivated at 350 rpm
3b: Average distribution of the plasmid isoforms open circular (OC) (green) and supercoiled (SC) (orange) isoform, measured with HPLC of the standard cultivation condition versus Thomson's Ultra Yield® system. Flasks were maintained at 350 rpm.

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Does Plasmid+® contain animal byproducts?

Thomson's Plasmid+® enriched media is manufactured without any animal byproducts and is an animal origin free formulation.

How was the Plasmid+® enriched media developed?

Thomson developed for a project with GNF (Thomson has no affiliation with Novartis). The Foundation's goal was to produce 1000 transients in 3 months, with LB this would not have been possible. Thus, various formulas of enriched media were developed to achieve Plasmid+® and the optimal enriched media for processing.

Do I need to add anything else to my culture when using Plasmid+® enriched media?

Thomson's Plasmid+® enriched media comes sterile and ready to use. Add antibiotics or antifoam as needed.

What antifoam is recommended for my culture when using Plasmid+® enriched media?

Thomson recommends using Sigma-Aldrich® Antifoam 204 or J.T.Baker Antifoam C Medical Emulsion with a 1:5000 ratio to cell culture medium.

What volumes can I purchase Plasmid+® enriched media?

Thomson's Plasmid+® enriched media is currently offered in 1L bottles only. For different volumes inquiries, please contact customer service or your Thomson technical sales representative.

How long can Plasmid+® enriched media perform under production?

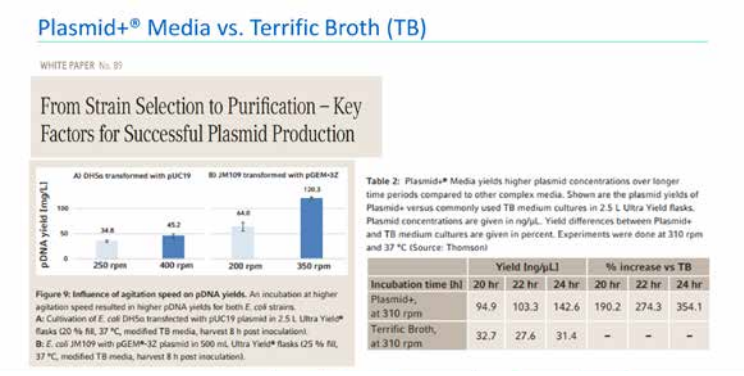
Thomson's Plasmid+® enriched media can support production for 24 hours without issue. LB broth will start to reduce in nutrients and cannot support growth after 16 hours.

Can low copy constructs grow with Plasmid+® enriched media?

Yes, pUC19 is a large plasmid and grew still better with Plasmid+® enriched media. Follow this link for the data: https://htslabs.com/technical/plasmid-dna-growth.

Can TB broth be replaced with Plasmid+® enriched media?

Thomson's Plasmid+® enriched media can be utilized for protein production within *E.coli* cells.


Plasmid+® Media vs. Terrific Broth (TB)

WHITE PAPER No. 89

From Strain Selection to Purification – Key Factors for Successful Plasmid Production

Figure 9: Influence of agitation speed on pDNA yields. An incubation at higher agitation speed resulted in higher pDNA yields for both E. coli strains.
A: Cultivation of E. coli DH5α transfected with pUC19 plasmid in 2.5 L Ultra Yield® flasks (20 % fill, 37 °C, modified TB media, harvest 8 h post inoculation).
B: E. coli JM109 with pGEM®-3Z plasmid in 500 mL Ultra Yield® flasks (25 % fill, 37 °C, modified TB media, harvest 8 h post inoculation).

Table 2: Plasmid+® Media yields higher plasmid concentrations over longer time periods compared to other complex media. Shown are the plasmid yields of Plasmid+ versus commonly used TB medium cultures in 2.5 L Ultra Yield flasks. Plasmid concentrations are given in ng/µL. Yield differences between Plasmid+ and TB medium cultures are given in percent. Experiments were done at 310 rpm and 37 °C (Source: Thomson)

	Yield [ng/µL]			% increase vs TB		
Incubation time [h]	20 hr	22 hr	24 hr	20 hr	22 hr	24 hr
Plasmid+, at 310 rpm	94.9	103.3	142.6	190.2	274.3	354.1
Terrific Broth, at 310 rpm	32.7	27.6	31.4	-	-	-

Does Plasmid+® enriched media come in a GMP controlled version?

Thomson can provide a made to order GMP Plasmid+® enriched media. For GMP inquiries, please reach out to customer service or your Thomson technical sales representative.

Recommended Growth Volumes with Plasmid+®

	Recommended Volume	Recommended Vessel
MINI Prep	0.5-1mL	Thomson 96-Well plate
MIDI Prep	4-5mL	Thomson 24-Well plate
MAXI Prep	35-50mL	125mL Ultra Yield® Flask
MEGA Prep	75-100mL	250mL Ultra Yield® Flask
	150-200mL	500mL Ultra Yield® Flask
GIGA Prep	300mL	1.5L Ultra Yield® Flask
GIGA Prep	500mL	2.5L Ultra Yield® Flask

Table 1: Recommended Growth Volumes with Plasmid+®

Troubleshooting

Low Protein Yield

- 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 350 RPM
- Increase the growth time (for up to 24 hours)
- Use a starter culture for final culture volumes > 50 mL
- Protein may be toxic. Try growth at 16 °C. Growth time may need to be increased at 16 °C

Low Recovery From Purification

- Make sure resuspension of cell pellet is complete
- Use enough resin for higher quantity yields

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