



**ULTRA YIELD® FLASKS,
PLASMID+®, AIROTOP™ SEALS**
THE *E. COLI* & MICROBIAL GROWTH SOLUTION



Thomson Ultra Yield® Solution

Thomson's Ultra Yield® Flask (patented) have proven over the last decade to enhance the growth of *E.coli* & other microbial cells. The patented flask design makes them the workhorse of protein and DNA labs worldwide. The Ultra Yield® Flasks come in standardized sizes of 125mL, 250mL, 500mL, 1.5L, & 2.5L.

The flasks are designed to be closed on top by

using our AirOtop™ Enhanced Seals (patented) or the new Vent Cap. The seals are designed to fit on the tops of the flasks. AirOtop™ Enhanced Seals are sterile, easy to use, and single use. The AirOtop™ Enhanced Seals properties include a 0.2µm resealable sterile membrane barrier providing high air exchange for all types of shake flasks. Multiple sizes are available to keep all of your flasks covered. The sterile

0.2µm PTFE Vent Caps have screw on to the 1.5L & 2.5L Ultra Yield® Flasks. Testing has been conducted at multiple customer sites with great results on up to 24 hours of growth. The organisms tested included Protista (Algae), *E.coli* and other microbes which have resulted in improved cell density, a more neutral pH of the cultures with increased gas exchange.



Ultra Yield® Flasks



Plasmid+® Media



AirOtop™ Enhanced Seals



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Ultra Yield® Flasks

Ultra Yield® Flasks enhance growth of *E.coli* and other microbial cells. The patented design enables increased cell growth.

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Plasmid+® Media

PLASMID+® is an enriched medium specifically designed to increase plasmid DNA and Protein production.

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AirOtop™ Enhanced Seals

AirOtop™ Enhanced Seals are simple to use, sterile, high air exchange, disposable seals for all shake flasks with a resealable membrane.



Key Features

- 10x increased aeration over standard shake flasks
- Increased DNA & protein production
- Fully scalable results
- Replacement for glass flasks
- Fits all standard flask clamps
- Easily adaptable into microbial growth protocols
- Sterile, disposable, single-use flasks from 125mL – 2.5L



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The flasks are designed to be closed on top by using our AirOTop™ Enhanced Seals. These seals are designed to fit on the tops of the flasks. AirOTop™ Enhanced Seals are sterile, easy to use, and single use. AirOTop™ properties include a 0.2µm re-sealable and sterile membrane barrier providing high air exchange for all types of shake flasks. Multiple sizes are available to keep all of your flasks covered. Testing has been conducted at multiple customer sites with great results on up to 24-hours of growth. The organisms tested included Protista (Algae), *E. coli*, and other microbes which have resulted in improved cell density, a more neutral pH of the cultures with the increased gas exchange.



Vent Caps are now available for Ultra Yield® Flasks

Recommended speeds:

Recommended culture volumes and orbital shaker speeds (a throw of 1" is generally used for orbital shaking):

Flask	Media (mL)/Flask	Shaker Speed
125mL	40-50mL/Flask	300-350 rpm
250mL	75-110mL/Flask	300-350 rpm
500mL	125-200mL/Flask	300-350 rpm
1.5L	250-350mL/Flask	300-350 rpm
2.5L	500mL (optimum) 500mL - 1L	300-400 rpm 250 rpm

How does the yield increase with Ultra Yield® Flasks?

The Ultra Yield® Flask increases yield. Because the Ultra Yield® Flasks allow increased cell densities to be achieved over traditional shake flask designs. This results in a concomitant increase in cell biomass leading to improved yields of recombinant protein and DNA. Cells in Ultra Yield® Flasks may be grown for more than 24 hours if necessary, as the cultures are not oxygen limited. Cells do not go directly from log phase to stationary phase but pass through a transition phase, as shown by data from GSK (see GSK data on page 7). This transition phase allows for continued growth without anaerobic conditions, and uses the cellular nutrients to maintain viability. pH maintenance is extremely important during this phase, buffering with a non-temperature dependant buffer, may make a large difference in cell growth behavior when using the Ultra Yield® Flask.

The high growth observed with the Ultra Yield® Flask has been found to be scalable across the different size variations, from the 125mL flask to the 2.5L flask. Studies have shown that the Ultra Yield® Flask provides consistent batch to batch results.

What are recommended Ultra Yield® Flask volumes and recommended orbital speeds for culturing *E.coli* and other microbes. The culture volumes deployed in Ultra Yield® Flasks is somewhat dependent on cell type for optimal performance. A general rule for *E.coli* is to use a 40% fill volume. End-users have deployed 500mL for maximum OD, or 1L for additional production in the same footprint. Often, 3-4 flasks may be used to replace a traditional small scale fermentor.

Is the Ultra Yield® Flask disposable or re-usable?

The Ultra Yield® Flasks are priced to be a disposable bioreactor or disposable shake flask. Even though Ultra Yield® Flasks are reusable, but the cost of the flasks is significantly cheaper than the autoclaving. We recommend using an Ultra Yield® Flask a maximum of three times because the plastic can become brittle and residual detergents can build up causing a drop off in density and lower yields. With costly labor costs, does it make sense to risk the experiment?

What is missing with LB and minimal media?

LB or Minimal Media do not contain sufficient nutrients to support microbial growth to high cell densities. The Ultra Yield® Flask cannot differentiate itself from traditional shake flask designs when using low growth media, and all flasks will perform in a similar fashion. However,

traditional shake flask designs cannot approach the cell densities that are achievable in the Ultra Yield® Flask.

Media choices and recommendations?

Different types exist, and by no means is this an endorsement of one over another: Plasmid+®, TB, Magic Media, Enbase, etc.

What orbital shaker equipment are suitable for use with the Ultra Yield® Flask?

Most shaker platforms support high density cell growth in the Ultra Yield® Flask, including those from New Brunswick, INFORS HT and Kuhner.

Myths and legends of growing cultures?

1. LB is a good media to use for microbial cultures: Ultra Yield® Flasks perform optimally when using an enriched media.
2. Growing cultures for more than 18 hours will damage my culture: What damages the growth and causes anaerobic growth is the insufficient amount of oxygen and drop in pH when using normal shake flasks. Ultra Yield® Flasks give sufficient aeration for 7 days of growth at 20°C, and 50 hours at 37°C.

What media do we put in the Ultra Yield® Flasks?

An enriched media is recommended with a balanced pH of approximately 7.4. TB, EnBase, Magic Media & Plasmid+® are all good examples of highly enriched medias. The pH balance is most useful with buffers that contain phosphate since these are not temperature dependent. ■

Ultra Yield® Flask Standard Protein Protocol

Description

The patented Ultra Yield® Flask is a unique disposable shake flask design that supports high-density culture growth in enriched media. The Ultra Yield® Flask achieves this by using a novel near-vertical wall angle coupled with a six baffle design that has been optimized for the fermentation of *E.coli*, *P.pastoris*, *S.pombe*, *Schizomycetes*, and other microbes. The Ultra Yield® Flask generates up to ten times greater gas exchange compared to traditional shake flask. The AirOtop™ Enhanced Seals and Vent Caps are used to cover the shake flasks preventing potential culture contamination. The Ultra Yield® Flask provides scalable culture growth. The Ultra Yield® Flasks are available in the following sizes: 125mL, 250mL, 500mL, 1.5L or 2.5L.

Bacteria strains

E.coli, DH5α, BL21, Rosetta, Orgami, etc. are compatible with the Ultra Yield® Flasks and AirOtop™ Enhanced Seals.

Seed culture

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 create a seed culture, grow up 50mL of the microbe of choice in a 250mL Ultra Yield® Flask with Enhanced AirOtop™ Seal: 50mL working volume. The overnight culture should be grown for at least 16 hours, at a temperature of 37°C at 350rpm (25mm or 1" Throw). This should be done in an orbital shaker using flask clamps. The 50mL working volume will then seed a 2.5L Ultra Yield® Flasks. Please note that 1:5000 of anti-foam should be used for limiting foaming of the culture.

Overnight Flask Production

Fill the Ultra Yield® 2.5L Production Flask to a working volume range of 500mL to 1L. This should consist of an enriched medium like Plasmid+® with buffer pH 7.0 - 7.4. Determine the density of the culture by measuring the Optical Density (OD @ 600nm) can be verified using a spectrophotometer.

The overnight flask should be at an OD of at least 13.0 on the low end with the high end OD being observed in the 25-35 range. This will ensure that your stock for your production flask will be both viable and healthy.

Induction of the Ultra Yield® Flasks

The cultures should be ready for induction after approximately 4 hours of agitation at 37°C or when the Optical Density reads 4 (@ 600nm). At this point the culture is in its log growth phase and is ideal for starting expression. Induction should then take place using your expression reagent given your specific construct.

To maximize production, the temperature should be dropped to 18-25°C with shake speeds of 300-350rpm for 500mL 250-275rpm for 1L.

Harvesting of the Ultra Yield® Flasks

At the end of the 24 hour growth cycle, remove the Ultra Yield® Flasks from the shaker and take final measurements of your culture. Adjust the pH as needed and begin the purification process; by disruption, spinning down and lysis the cell pellet depending on the purification protocol. ■

Ultra Yield® Flask (Simple Disposable Bioreactor) & Economical parallel protein expression screening: Scale-up in *E.coli*, Yeast, & Microbes

Sam Ellis¹, Mark Ultsch², Sandrine Dessoy³, Ciarán N. Cronin⁴

¹ Thomson Instrument Company www.htslabs.com

² Genentech 1 DNA Way South San Francisco, CA 94080

³ GSK Biologicals Rue de l'Institut 89 Rixensart 1330 Belgium

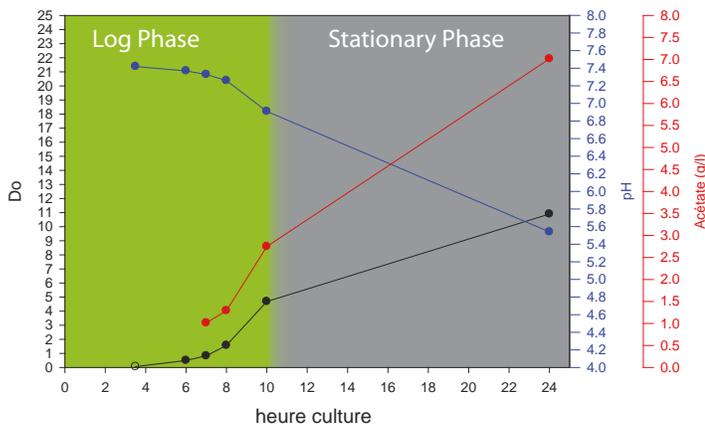
⁴ Pfizer, Inc., 10777 Science Center Drive, San Diego, CA 92122

Abstract

The patented Ultra Yield® Flask is a novel, disposable shake flask design that supports high-density culture growth in rich media. The Ultra Yield® Flask achieves this by using a novel near-vertical wall angle coupled with a six baffle design that has been optimized for the fermentation of *E.coli*, *P. pastoris*, *S.pombe*, *Schizomycetes*, and other microbes. Under suitable conditions, the Ultra Yield® Flask generates up to ten times greater oxygenation compared to traditional shake flask designs. A companion AirOtop™ air-porous seal is used to cover the flask from potential culture contamination, rather than traditional cotton plugs or aluminum foil. The Ultra Yield® Flask provides scalable culture growth results when using the 125mL, 250mL, 500mL, or 2.5L design.

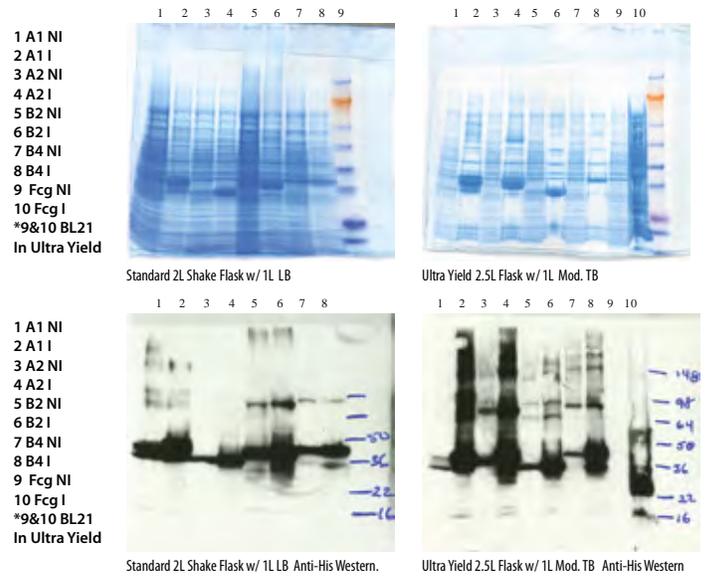
Conclusion

The Ultra Yield® Flask offers a simple alternative to traditional shake flasks, and to complex small-scale (<10L) fermentation devices at minimal cost. The Thomson Instrument Company, together with users at Genentech, GSK, and Pfizer, have validated the Ultra Yield® Flask as a suitable system to achieve high-density cell fermentation. Pfizer scientists have demonstrated that the Ultra Yield® Flask, on average, allows greater cell densities to be achieved in a similar footprint by up to 350%, and that this was mirrored by a similar return, on average, of 610% in recombinant protein yields (Brodsky, O. & Cronin, C.N. [2006]



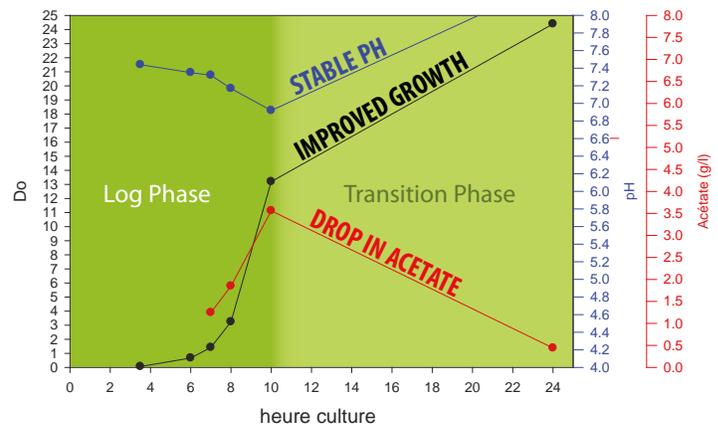
E.coli : Growth profiles **with classical glass flask** (2 L, no baffle),
400 mL Thomson medium, 400 rpm orbital shaker

Genentech
A Member of the Roche Group



J. Struct. Funct. Genomics. 7, 101-8). These data demonstrate that the Ultra Yield® Flask substantially reduces the protein production footprint, and may make traditional fermentation approaches unnecessary at smaller scales.

Fully automated fermentor systems available in the market today range from \$15,000 to \$90,000 dollars. However, the simple and inexpensive Ultra Yield® Flask solution can slash that price to only a few dollars which fits in any large or small research budget. Apart from the obvious fiscal advantage, other advantages include the ease of use (no requirement for detailed fermentation set-up and sterilization runs), the fitting of the flasks in standard laboratory floor shaker systems, and the simplicity of construction. It is anticipated that the Ultra Yield® Flask will become the flask of choice for recombinant protein production in the research setting.

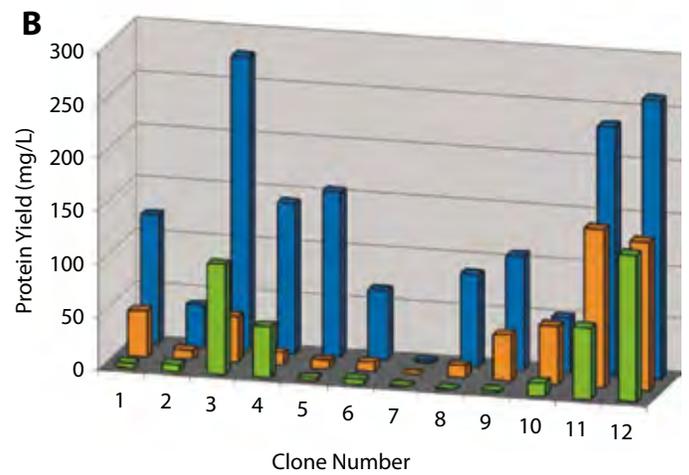
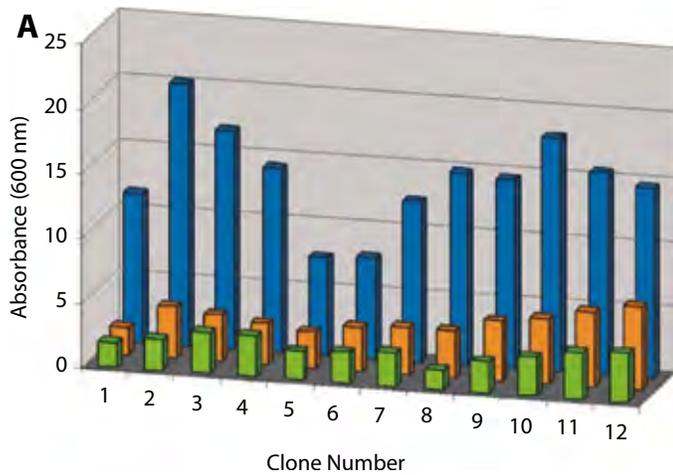


E.coli : Growth profiles **with Thomson flask** (2.5L Ultra Yield Flask, baffles), 500 mL Thomson medium, 400 rpm orbital shaker

—●— O.D. —●— pH —●— Acetate g/L

610% Yield Increase*

Effect of flask design on *E.coli* culture growth and production of recombinant protein. (A) The effect of flask type and growth medium on the observed optical densities of the cultures at A600 following overnight protein expression. Cultures carried out in Fernbach flasks using either LB medium or TB medium are shown, respectively, at the front (green) and in the center (orange). Cultures grown in Ultra Yield® flasks in TB medium are shown at the back (blue). (B) The yields of expressed soluble protein (determined by protein assay and recorded as mg protein per liter of expression culture) from the IMAC columns following purification of the twelve polyHis-tagged recombinant proteins, each expressed under the three conditions described in Panel A. Clone locations in Panel B are the same as those in Panel A ■



■ Thomson UYF
 ■ Corning®
 ■ Glass

Thomson Instrument Company is not affiliated with GSK, Genentech (a fully owned Roche company), Pfizer Inc, Kuhner AG, Corning, and New Brunswick Scientific.



CAUTION: Avoid ingestion and contact with skin. For lab use only. Not for drug, household or other uses. Certificate of Analysis, MSDS available upon request.



Key Features

- Medium specifically formulated for high DNA growth
- Increased supercoiled DNA per Liter
- Consistent plasmid production up to 22 hours
- Use with tubes, plates, flasks, and fermentors
- Sterile and ready to use. Simply add antibiotics and Grow!

PLASMID+

PLASMID+® is an enriched liquid medium specifically designed for plasmid DNA production.

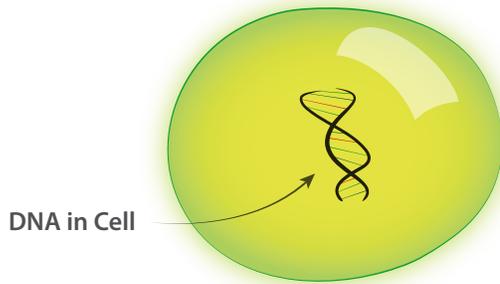
PLASMID+® supports higher cell densities and plasmid yields than LB media. Maximum culture aeration is achieved using the Ultra Yield® Flasks.

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

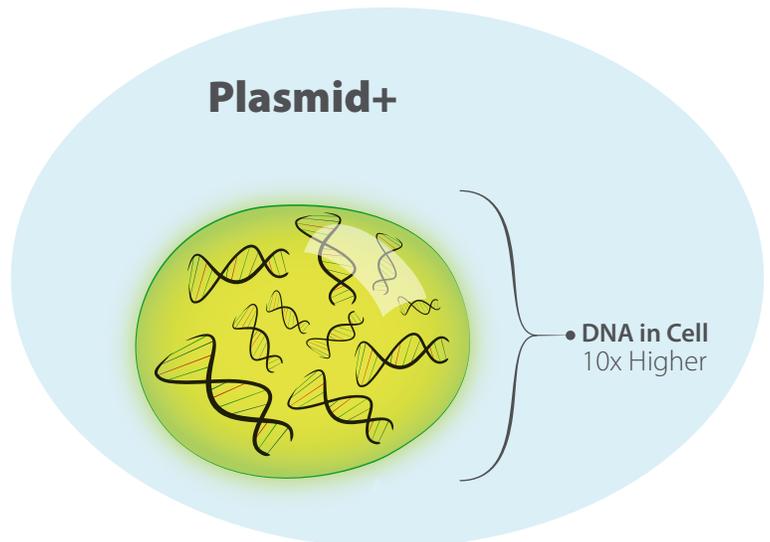


Other Broths

(LB, Circlegrow®, ect...)



Plasmid+



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	500-1000mL	1.5L or 2.5L Ultra Yield® Flask

Plasmid+® Media Tips

- Plasmid+ increases yield in DNA by supporting growth of a larger number of cells (higher density/OD).
- In order for this to happen ensure proper aeration by using highest shake speeds possible.
- For large scale DNA preps, use a 1mL aliquot with a MINI-prep to gauge DNA yields.

DNA ENRICHED CELL PASTE

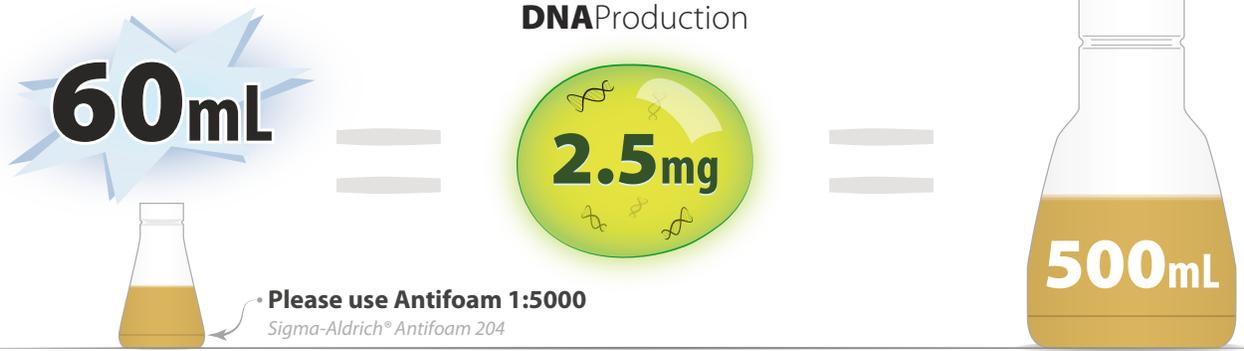
DON'T OVERLOAD YOUR COLUMN BINDING SITES



REDUCE CELL PASTE

< 2g CELL PASTE FOR MEGA PREP

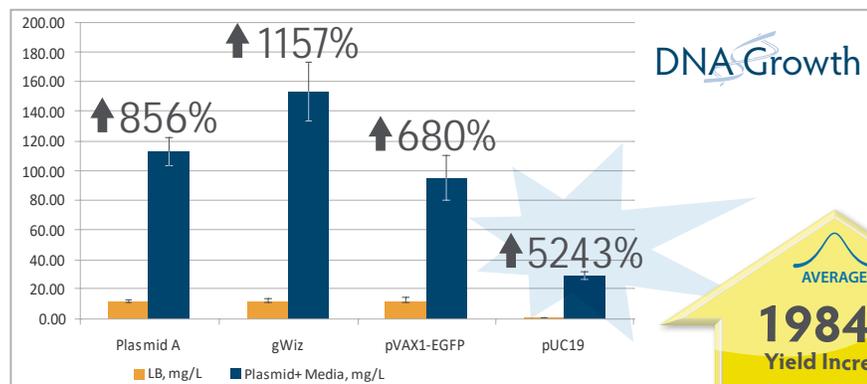
< 5g CELL PASTE FOR GIGA PREP



Plasmid+MEDIA

OTHER MEDIA

Thomson Ultra Yield® Flask for DNA Growth



Data for this graph can be found on our website htslabs.com in the Technical Library

...My plasmid maxi preps yield has improved a lot. I use Qiagen® Hi-Speed maxi kits and I used to grow 250mL bacterial culture to load on one column and my purified yield would barely be around 1mg/mL. Using Plasmid+® media I grow 50mL culture for one Qiagen® Hi-Speed column and in the past few weeks I even scaled the culture to 30mL/column and my yield is between 1-1.5mg/mL.

Novartis®, San Diego

Step 1



MINI-Prep

Determine DNA Concentration (mg/L)



Important Adjustments

- Please resuspend the cell pellet using **10mL of P1 buffer per gram** of cell pellet
- If preferred, use a volume of P1 buffer equivalent to half of the Plasmid+® culture volume is acceptable

Step 2



MEGA COLUMN

- ≤ **2grams of cell paste**
- 50-75mL of culture
- 5mg of DNA

GIGA COLUMN

- ≤ **5grams of cell paste**
- 400-450mL of culture
- 20mg of DNA



Maximum Culture Volume per Maxiprep is 30mL

Prep Procedure	Media	Culture Volume (mL)	Harvest OD	Cell Pellet weight (g)	HC-x DNA Yield (µg)
Manual	P+	25	4.4	0.7	861
Manual	P+	25	3.9	0.8	1140
Manual	P+	50	4.7	0.8	1125
Manual	LB	100	2.1	0.5	694

Troubleshooting

Low DNA 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 350 rpm • Confirm that an enriched media was used, such as Plasmid+® Media • 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 - don't overload your column!



Key Features

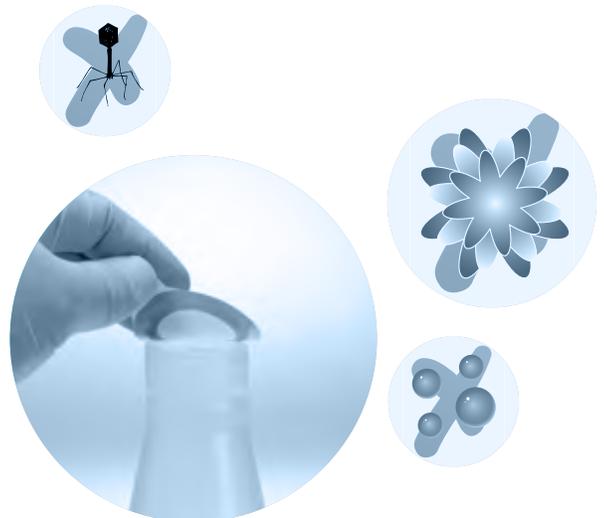
- 0.2µm high gas exchange flask seal
- Increased aeration
- Hydrophobic seal
- Resealable seal (tested up to 24 hours of growth)
- Compatible with standard flasks
- Eliminates flask contamination

AirOtopTM

Enhanced SealTM

Thomson AirOtopTM Enhanced Seals

AirOtopTM Enhanced Seals (patented) are disposable, high gas exchange, sterile, simple seals for all shake flasks. Properties of the seal include a 0.2µm sterile barrier with a resealable membrane for use with Ultra Yield[®] Flasks as well as other brand flasks. Multiple sizes are available to work with most commercially available shake flasks. Testing has been conducted by multiple customers with great results of up to 24-hours of growth. Testing included Protista (Algae), *E. coli*, and other microbes, and resulted in improved growth and more aerobic growth for all lines tested. Testing also showed that the pH of the cultures was more neutral.

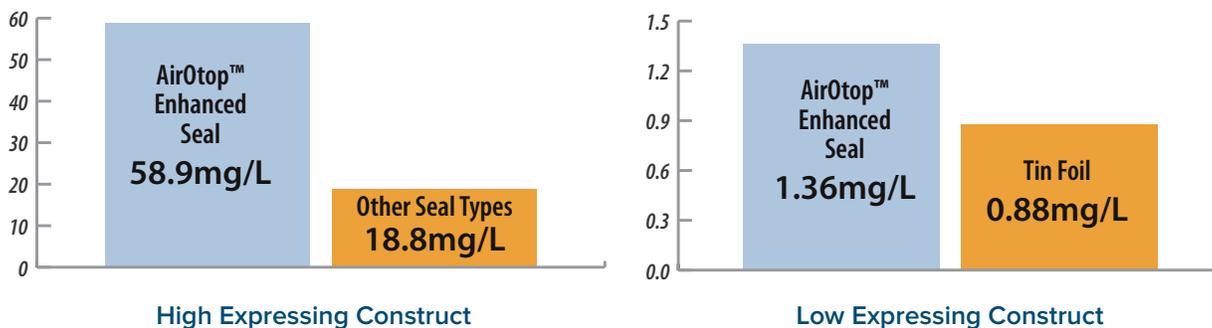


How do they work?

AirOtop™ Enhanced Seals can be applied as a clean, sterile barrier that allows samples to vent while preventing contamination from condensation and airborne particles such as phage, mold, spores, and dust. AirOtop™ Enhanced Seals replace plastic & metal flask caps, aluminum foil over flasks, cotton plugs, or cheese cloth. AirOtop™ Enhanced Seals are a convenient seal for many different types of flask. When used in combination with the Ultra Yield® flasks they become a simple “High yield production system”. The combination of Ultra Yield® Flasks and AirOtop™ Enhanced Seals allows maximum gas exchange improving production yields.



AiroTop™ Enhanced Seals Increase Expression



What about the seal I'm using now?

Common seals replaced with AirOtop™ Enhanced Seals

Seal Type	Sterile .2µm Air Barrier	High Aeration Rate	Disposable
AirOtop™	★	★	★
Metal Flask Cap			
Aluminium Foil			★
Cotton Plug			★
Cheese Cloth			★

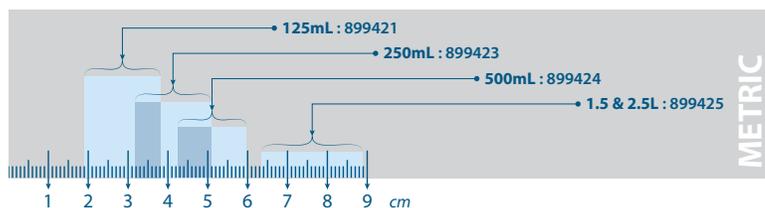
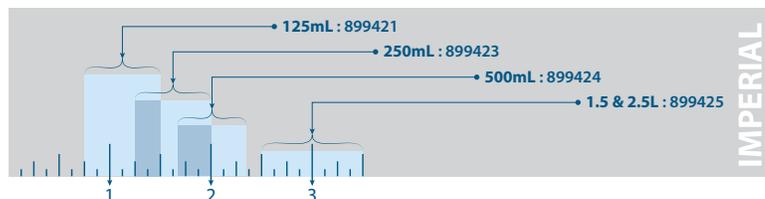




What about the flasks I'm using now?

AirOtop™ Enhanced Seal Compatibility

Thomson AirOtop™ Enhanced Seals are fully compatible with other manufacturer's flasks. Please see the compatibility charts (below) for identifying the correct seal for your flask.



AirOtop Seal	Nalgene-nunc®				
	125mL	250mL	500mL	1L	2L
899421	Compatible	Compatible			
899423			Compatible	Compatible	Compatible
899424					
899425					

AirOtop Seal	Corning®				
	125mL	250mL	500mL	1L	2L
899421	Compatible	Compatible	Compatible		
899423					
899424			Compatible	Compatible	Compatible
899425	Compatible	Compatible			Compatible

Thomson Instrument Company is not affiliated with Nalgene-nunc® or Corning Life Sciences® or their products



Key Features

- Bidirectional Transfer
- Fits 1.5L & 2.5L Ultra Yield® Flasks
- C-Flex 1/8" Tubing
- Sterile

ULTRA YIELD®

Bidirectional Transfer Cap for use with
1.5L & 2.5L Ultra Yield® Flasks

Part # 899136

How to Transfer or Feed

1. Screw on the Bidirectional Transfer Cap
2. Connect to your receiving vessel by Tube Fusing or using our Luer Lock option
3. Liquid can then be pumped either into or out of the flask



Part Numbers

Ultra Yield® Flasks			
Flask Size	125mL	250mL	500mL
Part #	931147	931144	931141
Image			
Description	Ultra Yield® 125mL Flask Sterile	Ultra Yield® 250mL Flask Sterile	Ultra Yield® 500mL Flask Sterile.
Seal Compatibility	AirOtop™ (899421)*	AirOtop™ (899423)*	AirOtop™ (899424)*
Flask Material	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)
Vented Screw Cap	n/a	n/a	n/a
Working Volume	40 - 50mL	75 - 110mL	125 - 200mL
Baffles	Yes	Yes	Yes
Qty/Case	50	50	25

Ultra Yield® Flasks			
Flask Size	1.5L	2.5L	2.5L
Part #	931138	931136-B	931136-B-VC
Image			
Description	Ultra Yield® 1.5L Flask Sterile	Ultra Yield® 2.5L Flask Sterile	Ultra Yield® 2.5L Flask w/ Vent Cap Sterile
Seal Compatibility	AirOtop™ (899425)*	AirOtop™ (899425)*	n/a
Flask Material	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)
Vented Screw Cap	n/a	n/a	0.2µm PTFE Vented Cap
Working Volume	250-350mL	500mL - 1L	500mL - 1L
Baffles	Yes	Yes	Yes
Qty/Case	12	6	6

Plasmid+® Media	
Part #	446300
Image	
Description	Plasmid+® Media in 1L Bottles Sterile
Final Product	Liquid Medium
Preparation Method	ready to use
Target Organism	<i>E. coli</i>
Product Size	1L
Qty/Case	6

* Sold separately

Transfer Cap	
Part #	899136
Image	
Description	Bidirectional Transfer Cap for 1.5L & 2.5L Ultra Yield™ Flask Sterile
Tubing Connection	Tube Fuse/Female Luer Lock
Tubing Diameter	C-flex 16 ID: 1/8" (3.1mm), oD: 1/4" (6.35mm)
Tubing Length	24" (609.6mm)
Top Style	Threaded
Cap Material	PP (polypropylene)
Sterility	10 ⁻⁶
Air Filter Ventilation	0.2µm PTFE vent filter
Qty/Case	6

AirOtop™ Enhanced Seal				
Flask Size Compatibility	125mL	250mL	500mL	1.5L & 2.5L
Part #	899421	899423	899424	899425
Image				
Description	AirOtop™ Enhanced Seal for Ultra Yield® 125mL Flask Sterile	AirOtop™ Enhanced Seal for Ultra Yield® 250mL Flask Sterile	AirOtop™ Enhanced Seal for Ultra Yield® 500mL Flask Sterile	AirOtop™ Enhanced Seal for Ultra Yield® 1.5L & 2.5L Flask Sterile
Qty/Case	100	100	100	100

Vent Cap				
Part #	899109	899110	899111	899566
Image				
Description	Vent Cap for 125mL Ultra Yield® Flask Sterile	Vent Cap for 250mL Ultra Yield® Flask Sterile	Vent Cap for 500mL Ultra Yield® Flask Sterile	Vent Cap for 1.5 & 2.5L Ultra Yield® Flask Sterile
Membrane Material	0.2µm PTFE	0.2µm PTFE	0.2µm PTFE	0.2µm PTFE
Qty/Case	50	50	25	24

000 «Диаэм»

Москва
ул. Магаданская, д. 7, к. 3 ■ тел./факс: (495) 745-0508 ■ sales@dia-m.ru

www.dia-m.ru

С.-Петербург
+7 (812) 372-6040
spb@dia-m.ru

Новосибирск
+7 (383) 328-0048
nsk@dia-m.ru

Воронеж
+7 (473) 232-4412
vrn@dia-m.ru

Йошкар-Ола
+7 (927) 880-3676
nba@dia-m.ru

Красноярск
+7 (923) 303-0152
krsk@dia-m.ru

Казань
+7 (843) 210-2080
kazan@dia-m.ru

Ростов-на-Дону
+7 (863) 303-5500
rnd@dia-m.ru

Екатеринбург
+7 (912) 658-7606
ekb@dia-m.ru

Кемерово
+7 (923) 158-6753
kemerovo@dia-m.ru

Армения
+7 (094) 01-0173
armenia@dia-m.ru

