

ULTRA YIELD™ FLASKS, PLASMID+®, AIROTOP™ SEALS

THE E.COLI & MICROBIAL GROWTH
SOLUTION



MADE IN USA

Thomson Ultra Yield™ Solution

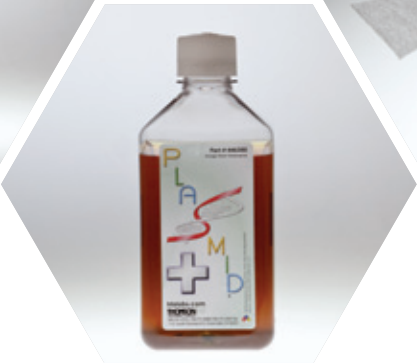
Thomson's Ultra Yield Flasks™ (patented) have proven over the last decade to enhance the growth of *E.coli* & other microbial cells. The patented flask design makes them the work horse of protein and DNA labs worldwide. The Ultra Yield Flasks come in standardized sizes of 125mL, 250mL, 500mL and 2.5L.

The flasks are designed to be closed on top by using our Enhanced AirOtop™ Seals (patented). These seals are designed to fit on the tops of the flasks. Enhanced AirOtop™ Seals are sterile, easy to use, and single use. The Enhanced AirOtop™ Seals properties include a 0.2µm resealable sterile membrane barrier providing high air exchange for all

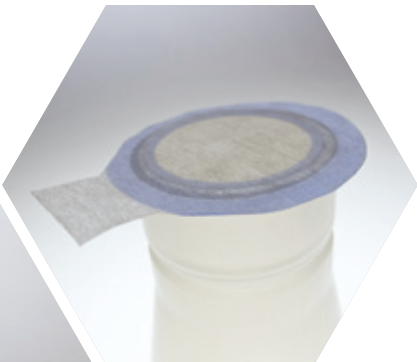
types of shake flasks. Multiple sizes are available to keep all of your flasks covered. Testing has been conducted at multiple customer sites with great results on up to 24 hours of growth. The organisms tested included Protista (Algae), *E.coli* and other microbes which have resulted in improved cell density, a more neutral pH of the cultures with the increased gas exchange.



Ultra Yield™ Flasks



Plasmid+ Media



AirOtop
Enhanced Seals



4



Ultra Yield™ Flasks

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

10



Plasmid+® Media

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

14



AirOtop™ Enhanced Seals

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



2.5L Ultra Yield™ Flask with 0.2µm PTFE Vent Cap (pn# 931136-B-VC)

- 10x Increased Aeration Over Standard Shake Flasks
- Increased DNA & Protein Production
- Fully Scalable Results
- Replacement For Glass Flasks
- Fit All Standard Flask Clamps
- Easily Adaptable Into Microbial Growth Protocols
- Sterile, Disposable, Single-Use Flasks From 125mL - 2.5L

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 Enhanced AirOTop™ Air Porous Seals are used to cover the shake flasks preventing potential culture contamination, rather than traditional cotton plugs or aluminum foil. The Ultra Yield™ Flask provides scalable culture growth results when using the Ultra Yield™ Flasks: 125mL, 250mL, 500mL, or 2.5L.

Bacteria strains

E.coli, DH5α, BL21, Rosetta, Orgami, etc. are all compatible with the Ultra Yield™ Flasks and Enhanced AirOTop™ 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+® or other enriched media 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 is 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 your 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*, *Schizomyces*, 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.

Materials and Methods

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

An enriched media with a pH balance around 7.4 is recommended. Plasmid+® and others are suitable examples of enriched media. The pH balance is most useful with buffers that contain phosphate since these are not temperature dependent. Personally we prefer media to be buffered.

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

The Ultra Yield™ Flask is priced to be a disposable bioreactor or disposable shake flask. The Ultra Yield™ Flask may be reused following sterilization. However, the cost of the flask is significantly cheaper than the effort required for autoclaving (with labor at ~ \$100.00/h it does not make sense to reuse the product?). Nevertheless, if one wishes to reuse the flask it is possible to do so a number of times. However, multiple autoclaving runs will make the flask more brittle and likely to

fail. A limit of three uses is suggested.

Myths and legends of growing cultures?

1. LB is the least healthy growth media. High density microbial growth will only be achieved in the Ultra Yield™ Flask when using an enriched media, such as Plasmid+®.
2. Growing cultures for more than 18h will damage the culture. Cell damage and inferior growth is generally the result of anaerobic conditions resulting from a lack of oxygen, and a drop in pH when using traditional shake flask designs. The Ultra Yield™ Flask provides sufficient aeration to maintain cell growth for up to 7 days at 20°C, and up to 50h at 37°C.

Why are LB and Minimal Media unsuitable for the Ultra Yield™ Flask?

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.

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.

How does the yield increase with Ultra Yield™ Flasks?

The Ultra Yield™ Flask improves yield. Because the Ultra Yield™ Flask allows increased cell densities to be achieved over traditional shake flask designs, this results in increased yields of biomass and concomitant improvement in yields of recombinant protein or 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). This transition stage allows for continued growth without anaerobic conditions, and uses the cellular nutrients to maintain viability. pH maintenance is extremely important during this phase, and 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.

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.

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
2.5L	500mL (optimum) 500mL - 1L	300-400 rpm 250 rpm

Conclusion

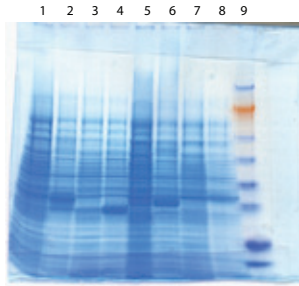
The Ultra Yield™ Flask offers a simple alternative to traditional shake flasks, and to complex small-scale (<10L) fermentation devices at minimal cost. The Thomson Instrument Company, together with users at Genentech, GSK, and Pfizer, have validated the Ultra Yield™ Flask as a suitable system to achieve 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] 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. ■

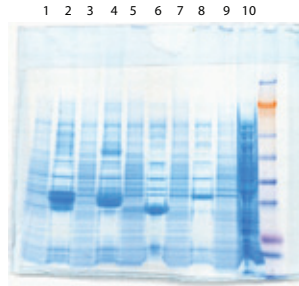


Thomson Instrument Company is not affiliated with GSK, Genentech (a fully owned Roche company), Pfizer Inc, Kuhner AG, Corning, and New Brunswick Scientific.

1 A1 NI
2 A1 I
3 A2 NI
4 A2 I
5 B2 NI
6 B2 I
7 B4 NI
8 B4 I
9 Fcg NI
10 Fcg I
*9&10 BL21
In Ultra Yield

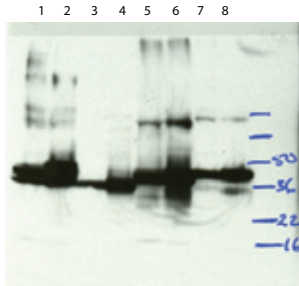


Standard 2L Shake Flask w/ 1L LB

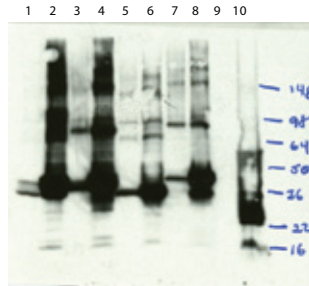


Ultra Yield 2.5L Flask w/ 1L Mod. TB

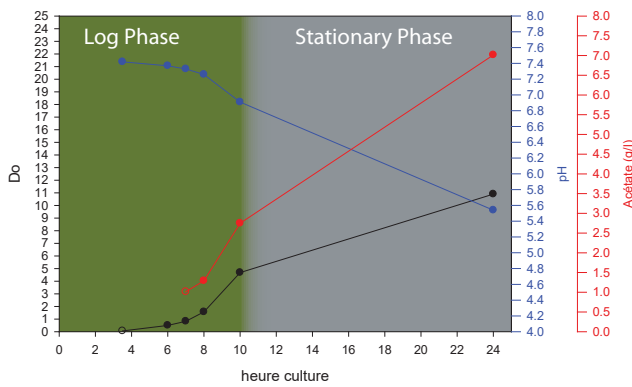
1 A1 NI
2 A1 I
3 A2 NI
4 A2 I
5 B2 NI
6 B2 I
7 B4 NI
8 B4 I
9 Fcg NI
10 Fcg I
*9&10 BL21
In Ultra Yield



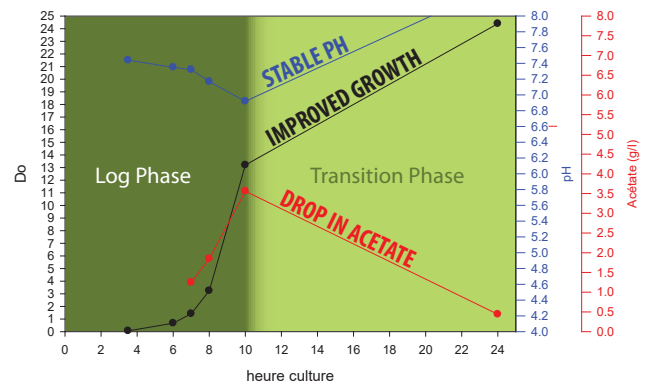
Standard 2L Shake Flask w/ 1L LB Anti-His Western.



Ultra Yield 2.5L Flask w/ 1L Mod. TB Anti-His Western

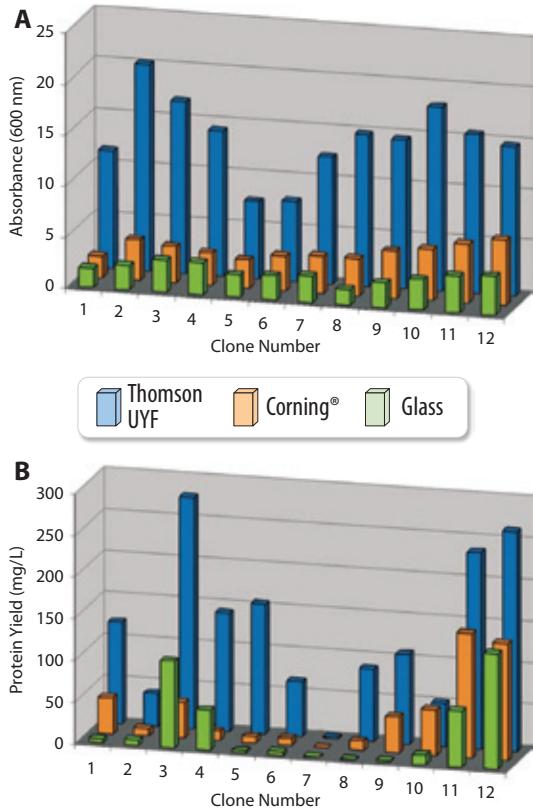


E.coli : Growth profiles **with classical glass flask** (2 L, no baffle), 400 mL Thomson medium, 400 rpm orbital shaker



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





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

*Economical parallel protein expression screening and scale-up in *Escherichia coli*. Journal of Structural and Functional Genomics 2006 Jun;7(2):101-8. Epub 2006 Dec 23.



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.



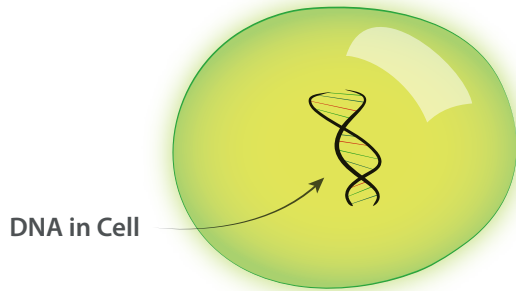
CAUTION: Avoid ingestion and contact with skin. For lab use only. Not for drug, household or other uses. Certificate of Analysis, MSDS available upon request.



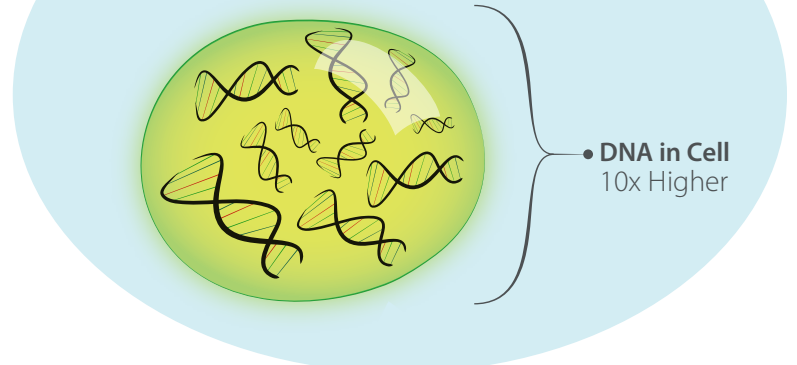
- Medium specifically formulated for high DNA growth
- Increased Super Coiled DNA per Liter
- Use with: mini-prep, midi-prep, maxi-prep, and giga-prep
- Eliminates repeated runs & media prep errors
- Consistent Plasmid production up to 22 hours
- Use with tubes, plates, flasks, and fermenters
- Sterile, ready to use. Simply add antibiotics and Grow!



Other Broths (LB, Circlegrow®, ect...)

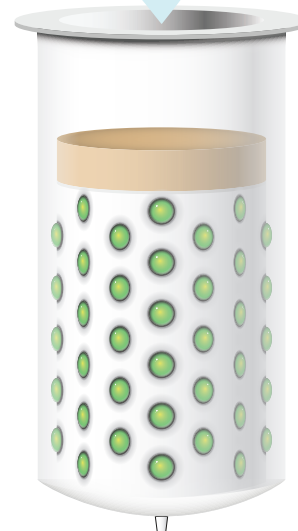


Plasmid+



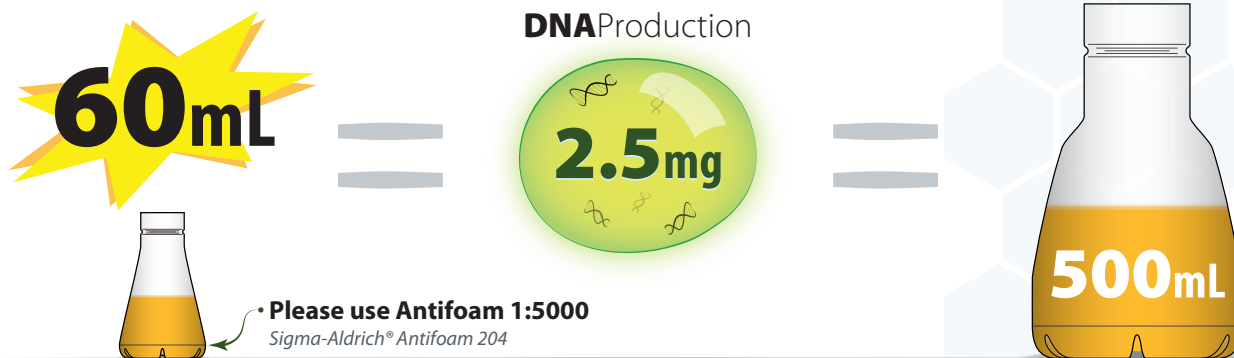
DNA ENRICHED CELL PASTE

DON'T OVERLOAD
YOUR COLUMN
BINDING SITES



**REDUCE
CELL PASTE**
< 2G CELL PASTE
FOR MEGA PREP
< 5G CELL PASTE
FOR GIGA PREP

Mega-Prep Comparison Using Plasmid+



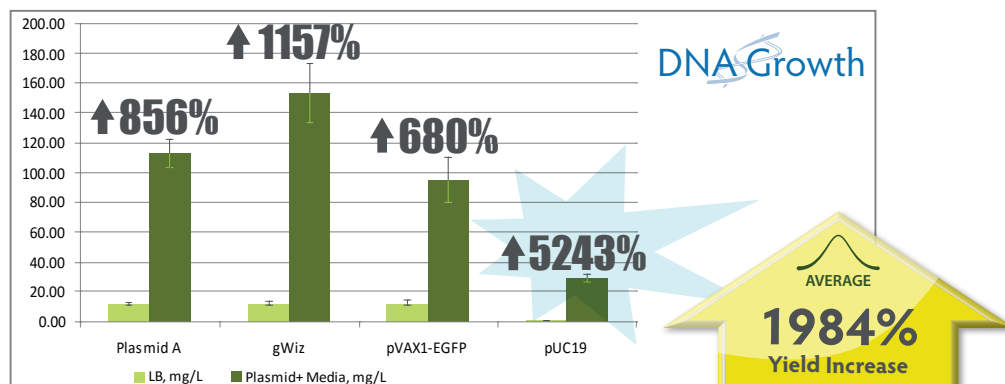
Plasmid+ MEDIA

OTHER MEDIA

...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

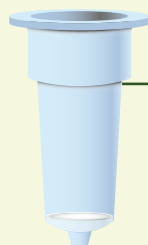
Thomson Ultra Yield™ Flask for DNA Growth



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

Plasmid+® Media: Maximum Column Loading

Step 1

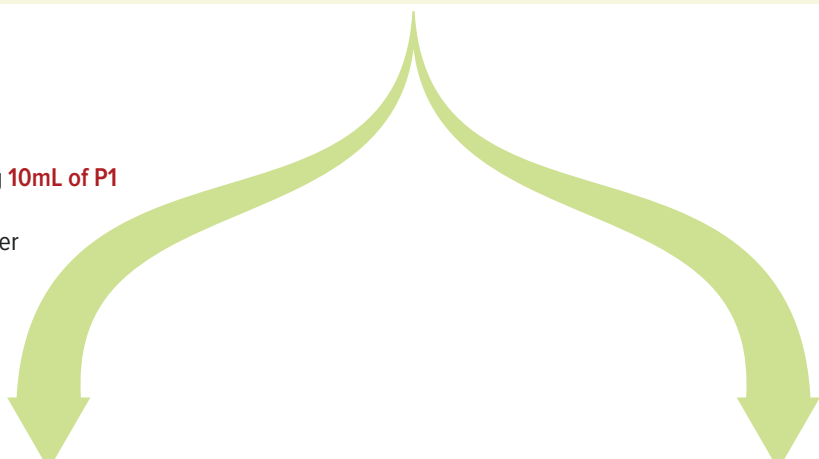


MINI-PREP

Determine DNA Concentration (mgs/L)

Important Adjustments

- Please resuspend 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



Step 2



MEGA COLUMN

- **≤ 2grams of cell paste**
- 50-75mLs of Culture
- 5mgs of DNA



GIGA COLUMN

- **≤ 5grams of cell paste**
- 400-450mLs of Culture
- 20mgs of DNA

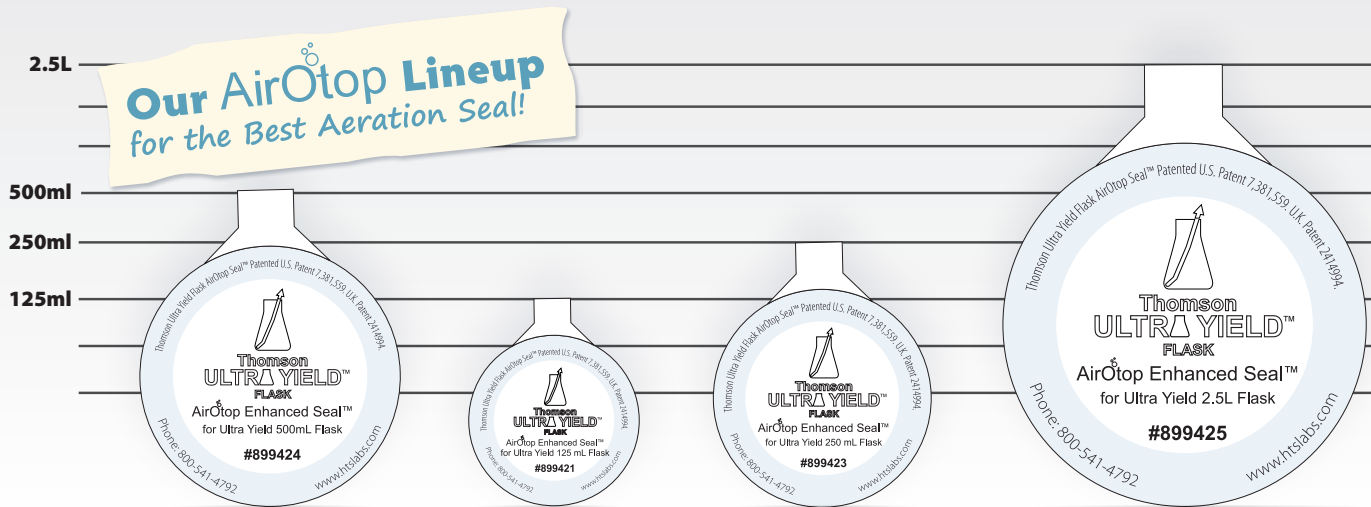
Troubleshooting

Low DNA 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 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

- Make sure resuspension of cell pellet is complete
- Use enough resin for higher quantity yields - don't overload your column!



Tin Foil
Can NOT Breathe



AirOtop
CAN Breathe



Enhanced AirOtop™ 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 customer with great results of up to 24 hours of growth. This testing included Protista (*Algae*), *E.coli* and other microbes which, has all resulted in improved growth and more aerobic growth. Also, showing that the pH of the cultures were more neutral.

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



Phage



Mold



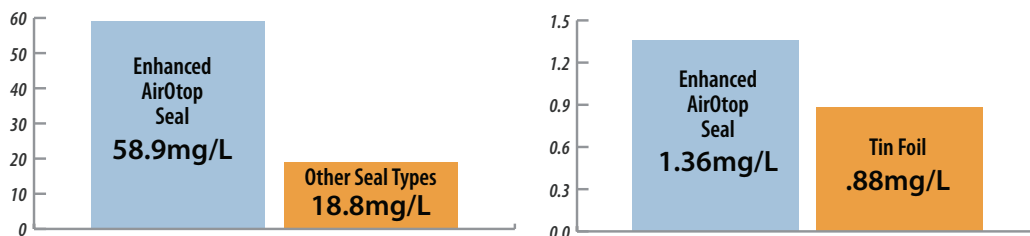
Spores

How do they work?

Enhanced AirOtop™ 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. Enhanced AirOtop™ Seals replace plastic & metal flask caps, aluminum foil over flasks, cotton plugs, cheese cloth, etc. Enhanced AirOtop™ Seals are a convenient seal for all different flasks. When used in combination with the Ultra Yield™ Flasks they become a simple “High Yield Production System”. Using the combination of Ultra Yield™ Flasks and Enhanced AirOtop® Seals yield maximum gas exchange improving production yields.



AirOtop™ Seals Increase Expression



What about the seal I'm using now?



Common seals replaced with Enhanced AirOtop Seals

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

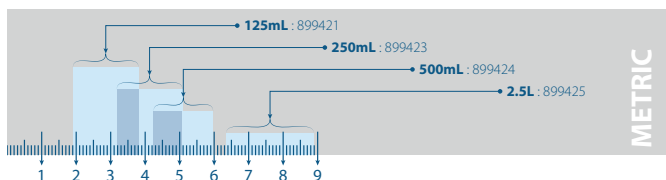
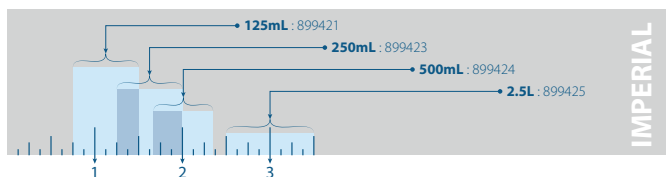


What about the flasks I'm using now?



Enhanced AirOtop™ Seal Compatibility

Thomson Enhanced AirOtop™ 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			
899423			Compatible		
899424				Compatible	
899425					Compatible


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

Part Numbers

Ultra Yield™ Flasks					
Flask Size	125mL	250mL	500mL	2.5L	2.5L
Part #	931147	931144	931141	931136-B	931136-B-VC
Image					
Description	125mL flask, 40-50mL working Volume - Sterile	250mL flask, 75-110mL working Volume - Sterile	500mL flask, 125-200mL working Volume - Sterile.	2.5L flask, 500mL-1L working Volume - Sterile	2.5L flask w/Vent Cap, 500mL-1L working Volume - Sterile
Seal Compatibility	AirOtop™ (899421)*	AirOtop™ (899423)*	AirOtop™ (899424)*	AirOtop™ (899425)*	n/a
Flask Material	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)
Vented Screw Cap	n/a	n/a	n/a	0.2µm PTFE Vented Cap*	0.2µm PTFE Vented Cap
Working Volume	40mL - 50mL	75mL - 110mL	125mL - 200mL	500mL - 1L	500mL - 1L
Baffles	Yes	Yes	Yes	Yes	Yes
Qty/Case	50	50	25	6	6

* Sold separately

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

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

Accessories	
Part #	899566
Image	
Description	Vent Cap for 2.5L Ultra Yield™ Flask - Sterile
Membrane Material	0.2µm PTFE
Qty/Case	24



35110-TC-K