



Cell Culture Solutions Ultra Yield® System

Microbial & *E. coli* Cell Growth



Ultra Yield® System

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An Introduction to the Ultra Yield® System

The Ultra Yield® System consists of three components: Ultra Yield® Flasks, AirOtop™ Enhanced Seals and Plasmid+® Enriched Media. Superior results are achieved when all three components are used simultaneously.



Ultra Yield® Flasks

Enhance the growth of *E. coli* and other microbial cells up to 10x over traditional flasks. Available in standardized volumes of 125mL, 250mL, 500mL, 1.5L and 2.5L



Plasmid+® Media

Is animal product-free media and adds all the essential nutrients required to support the enhanced cell growth obtained by using Ultra Yield® Flasks



AirOtop™ Enhanced Seals

As an alternative to foil or cotton plugs Air-O-top Seals provide a consistent and sterile gas-permeable barrier. Note that Vented Screw Caps are also available for Ultra Yield flasks



Ultra Yield® Flasks

Thomson's Ultra Yield system has proven over the last decade to enhance the aeration of *E. coli* and other microbial cells.

Ultra Yield Flasks are designed to be either single-use or autoclaved up to 3x and feature a unique geometry that enhances gas exchange.



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, autoclavable flasks from 125mL – 2.5L
- Use with AirOtop™ Enhanced Seals or Vented Screw Caps & Plasmid+® media

Ultra Yield Flask

FAQ's & End User Questions

Why do Ultra Yield® Flasks work better than glass flasks, and other disposable baffled flasks for *E. coli*, *Pichia* and *Schizosaccharomyces pombe*?

Ultra Yield® Flasks (patented) are a disposable high aeration shake flask or bioreactor. Ultra Yield® Flasks achieve high aeration with a 6 baffle design that has been optimized for fermentation for *E. coli*, *Pichia*, *Schizosaccharomyces pombe*, and other microbes. They provide good airlift with high speed shaking giving an increase of 10-fold oxygenation. When combined with nutrient enriched media and proper pH balance Ultra Yield® Flasks can increase yield significantly. No cutting of aluminum foil or exposure to cross contamination with used flask plugs. Ultra Yield® Flasks allow for scalable results from the 125mL, 250mL and 500mL up to the 1.5L and 2.5L flask volumes.

How does protein or DNA yield increase with Ultra Yield® flasks?

The Ultra Yield® Flasks act as a bioreactor to grow more biomass or cells per unit volume compared with traditional shaker flasks. With an increase in density there comes an increase in protein or DNA yield. Because they are not oxygen limited microbial cells in Ultra Yield® Flasks can be grown for over 24 hours. With regard to cell growth microbial cells in Ultra Yield® Flasks do not go from log phase to stationary phase rather, as shown by data from GSK, go to a transition phase. This transition phase allows for continued growth without the anaerobic conditions encountered in traditional flasks that use up the cells' own nutrients. With Ultra Yield® Flasks while pH maintenance is extremely important, buffering with a non-temperature dependent buffer like MOPS can make a large difference in cell growth. Ultra Yield® Flasks allow for superior growth in shake flasks, and scalable results from the 125mL to 2.5L flask.

What ratio of liquid media can we have in Ultra Yield® Flasks for *E. coli* and microbes? What are the recommended shake speeds for *E. coli* and microbes?

Media ratio depends on the cell type being grown. A general rule for *E. coli* is to use a 40% fill volume. For example, in the 2.5L Ultra Yield® Flask for maximum OD, we recommend using between 500mL and 1L of media. Three to four 2.5L Ultra Yield® Flasks will replace a small-scale fermenter.

Fill Volumes & Shake Speeds:

Recommended culture volumes & 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 -1L	250-400 rpm

Has anyone tested Ultra Yield® Flasks for acetate concentration? Can you use it as a starter culture for vaccines?

GSK has kindly provided data on pH, OD, and acetate parameters during *E. coli* growth in both traditional shake flasks and Ultra Yield® Flasks. These studies show that while OD rapidly increases pH remains stable and acetate levels actually decrease over time with Ultra Yield® Flasks. However, in traditional flasks OD increases more slowly while pH drops and acetate levels increase over the same time period.

What clamps and shakers work best for the Ultra Yield® Flasks?

Ultra Yield® Flasks are designed to shake in 1 inch orbit shakers at high speeds. Higher speeds allow for better aeration, however, at higher speeds it is necessary to use flask clamps rather than sticky tape. Our 125mL, 250mL and 500mL flasks will work with standard shake flask clamps. The 2.5L Ultra Yield® flask can fit into a standard 2L shake flask clamp. New Brunswick and Kuhner shakers have also made a special flask clamp. See our clamp cart below for specific clamp recommendations.

Flask Clamp Compatibility

Refer to the chart below to find the appropriate flask clamp compatible with your shaker.

Flask	Eppendorf®	Infors HT®	Kuhner®	Fisher Scientific®	VWR®
125mL	M1190-9001	12202 ATK 100	SM310100	11-676-013	57019-676
250mL	M1190-9002	12203 ATK 250	SM310250	11-676-014	57019-678
500mL	M1190-9003	12204 ATK 500	SM310500	11-676-015	57019-682
1.5L	ACE1000S	12205 ATK 1000	SM311000	11-676-016	57019-684
2.5L	M1190-9005	12206 ATK 2000	SM312500U	11-676-017	57019-686

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

Recommended media is an enriched media with pH balance around 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.

Are the Ultra Yield® Flasks disposable or re-usable?

This depends on your individual laboratory policies. Ultra Yield® Flasks are priced to be a disposable bioreactor or disposable shake flask even when also adding in the cost of autoclaving. Keep in mind that when reusing flasks a little residual detergent in the flasks and subsequent ruining of an experiment is more expensive than just using a new flask. However, Ultra Yield® Flasks are autoclavable and reusable. If you were to reuse the flasks we suggest not more than three rounds of autoclaving before disposal as polypropylene becomes brittle over time.

Myths and legends of growing cultures?

LB is a good media to use for my microbial cultures: Ultra Yield® Flasks perform optimally when using an enriched media like Plasmid+®.

Growing cultures for more than 18 hours will damage my culture: What slows down microbial growth and causes anaerobic growth to occur is an insufficient amount of oxygen and subsequent drop in pH when using traditional shake flasks. Ultra Yield® Flasks give sufficient aeration for up to 7 days of culture at 20°C, and up to 50 hours culture at 37°C.

What is missing with LB and minimal media?

LB or minimal media is like feeding a person bread and water. People need nutrients and vitamins. This is true with other living organisms such as microbes as well. We all need a balanced diet to grow strong.

What systems are people using with Ultra Yield® Flasks?

Scientists use Ultra Yield® Flasks on New Brunswick, INFORS HT, Kuhner, and every other shaking platform available.

End User Questions

We intend to autoclave Ultra Yield® Flasks and media between uses. Can Ultra Yield® Flasks and AirOtop™ Seals or Vented Screw Caps withstand autoclaving? Are flasks, seals and caps sterile and ready to use?

Ultra Yield® Flasks and AirOtop™ Enhanced Seals and Vented Screw Caps come ready to use sterile. It is acceptable to add media and sterilize the flask with the media in it, but do not use AirOtop™ Enhanced Seals or Vented Screw Caps during this process. Sterilization is best done with a piece of Aluminum foil to seal the top. After autoclaving allow the Ultra Yield® Flasks to cool down before using as they do retain heat. Microbes can be destroyed by adding them to flasks taken straight from the autoclave without letting them first cool to the proper temperature.

How do you grow your cells and media?

We usually prepare a large volume of media and sterilize it in bottles. Then pour the ready-made media into the Ultra Yield® Flasks and add the cells. We find this works best and is easier and faster for multiple runs.

When should I use a Vented Screw Cap vs an AirOtop™ Enhanced Seal?

Both Vented Screw Caps and AirOtop™ Enhanced Seals offer consistent sterile gas exchange for microbial cultures. AirOtop™ Enhanced Seals are resealable for up to 24 hours.

Thomson Instrument Company is not affiliated with TB, Magic Media, Enbase, New Brunswick, INFORS HT, Kuhner, GSK or their products



Plasmid+®

Enriched Media

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.

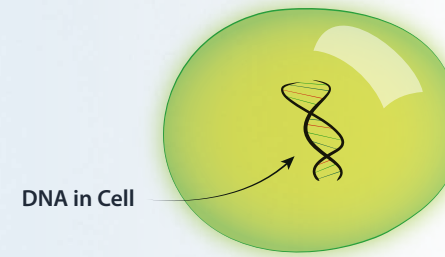
Key Features

- 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 fermenters
- Sterile and ready to use. Simply add antibiotics and Grow!
- Animal product-free formulation
- Store Plasmid+ liquid media at room temperature for up to 12 months



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.

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



Plasmid+



DNA Enriched Cell Paste Don't Overload Your Column Binding Sites

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.

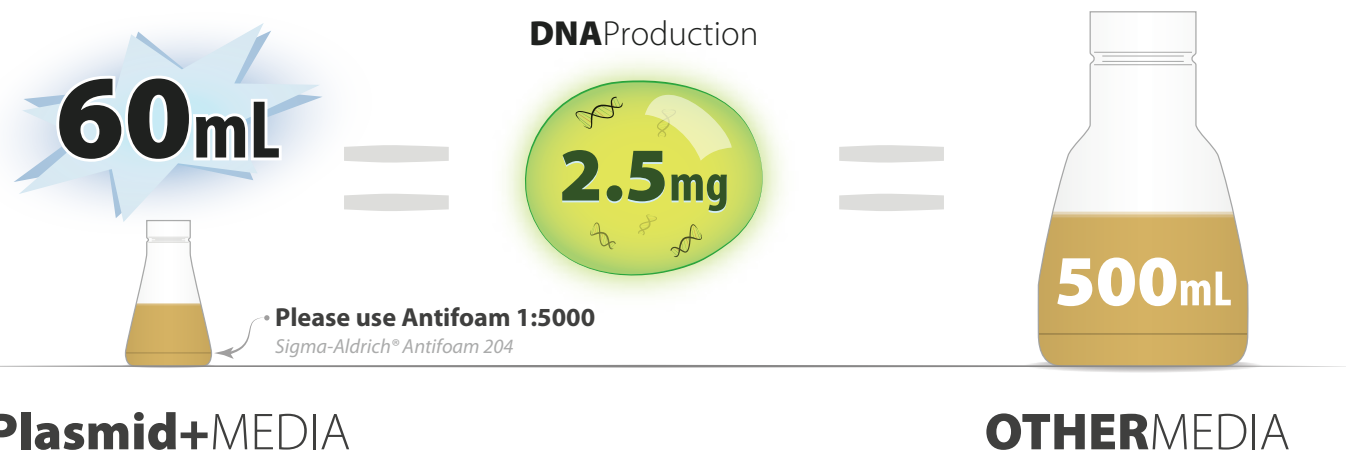
< 2g CELL PASTE
FOR MEGA PREP
< 5g CELL PASTE
FOR GIGA PREP

MEGA-Prep Comparision Using Plasmid+

Increased Efficiency

In Plasmid Production

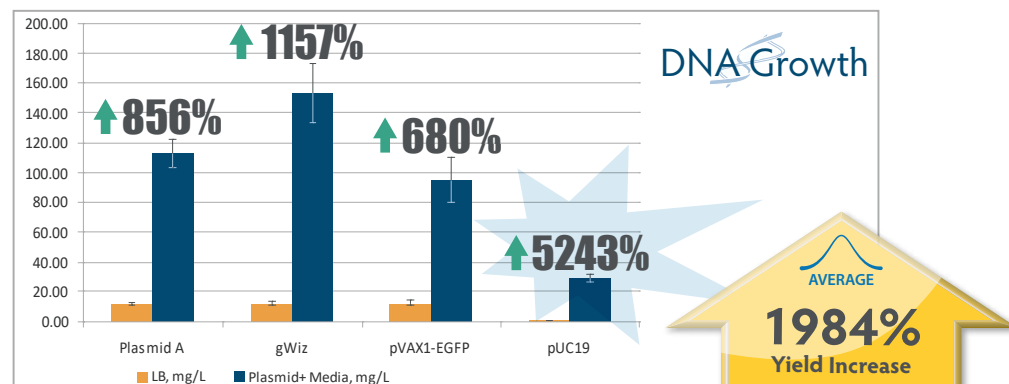
Increased efficiency in plasmid production when using Plasmid+ media over conventional media. A plasmid yield of 2.5mg was achieved when using either a 2.5L Ultra Yield® flask with 500mL of conventional media or a 250mL Ultra Yield® flask with 60mL of Plasmid+® media. No need to use large flasks and lots of media when you can get by with 8x less media to generate the same results.



Improved DNA Protocol

For *E.coli* With Plasmid+ Media

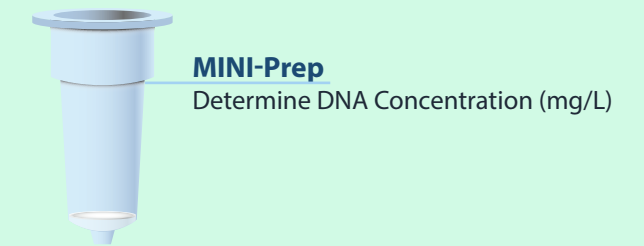
Four *E. coli* constructs were grown in either LB media or Plasmid+® media in 250mL Ultra Yield® Flasks using optimal fill volumes and shake speeds. After 24 hours in culture each flask was harvested and plasmid yields compared. As shown, Plasmid+® media greatly improved plasmid yields in all cultures on average over 1900% compared with LB Media



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

Plasmid+ Media Maximum Column Loading

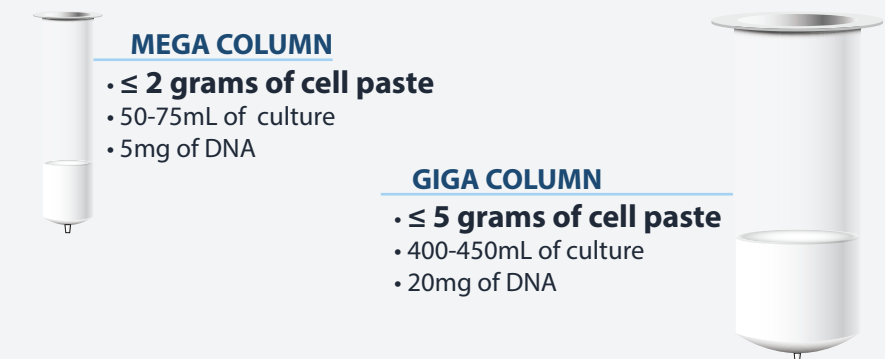
Step 1



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



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	<p>Check that the proper antibiotic and concentration is used</p> <p>Insure proper culture aeration. Use the recommended media volumes in Ultra Yield® Flasks with shaking at 350 rpm</p> <p>Confirm that an enriched media was used, such as Plasmid+® Media</p> <p>Increase the growth time (for up to 48 hours)</p> <p>Use a starter culture for final culture volumes > 50mL</p> <p>Protein may be toxic, try growth at 16°C. Growth time may need to be increased at 16°C</p>
Low Recovery From Purification	<p>Make sure resuspension of cell pellet is complete</p> <p>Use enough resin for higher quantity yields - don't overload your column!</p>

AirOtop™

Enhanced Seals

& Vented Screw Caps

As a component of the Thomson Ultra Yield System, AirOtop™ Enhanced Flask Seals are disposable, high gas exchange, sterile seals for all shake flasks.

AirOtop™ Enhanced Seals

AirOtop™ Enhanced Seals (patented) are disposable, high gas exchange, sterile seals for all shake flasks and are a component of Thomson's Ultra Yield® Solution and provide you with:

Key Features

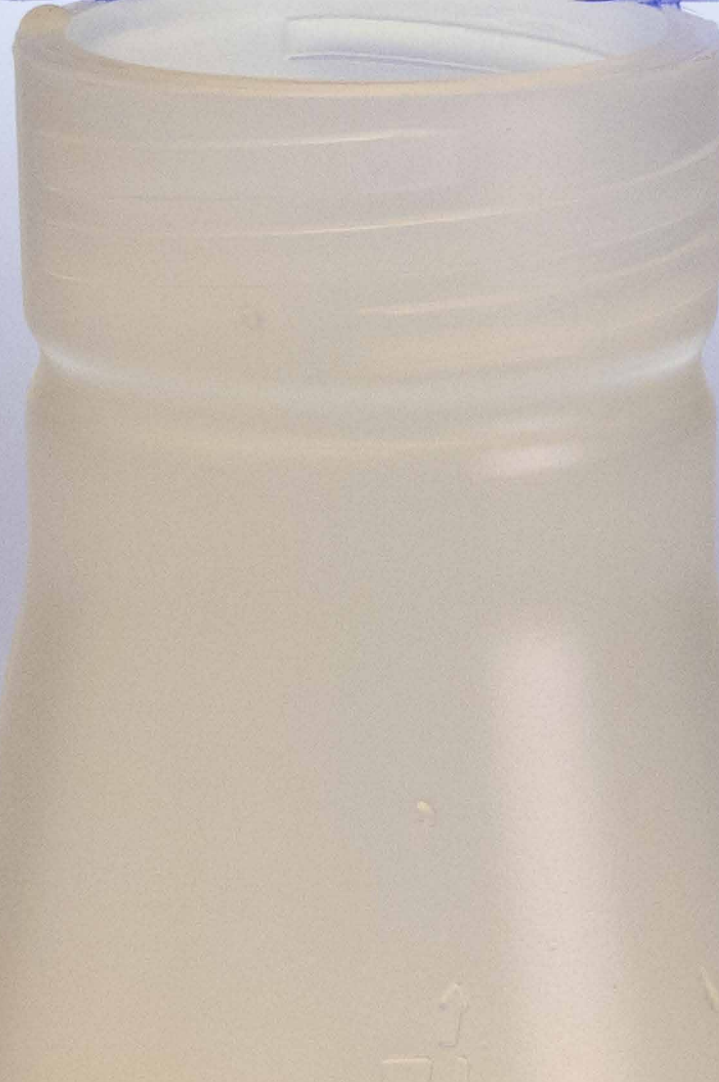
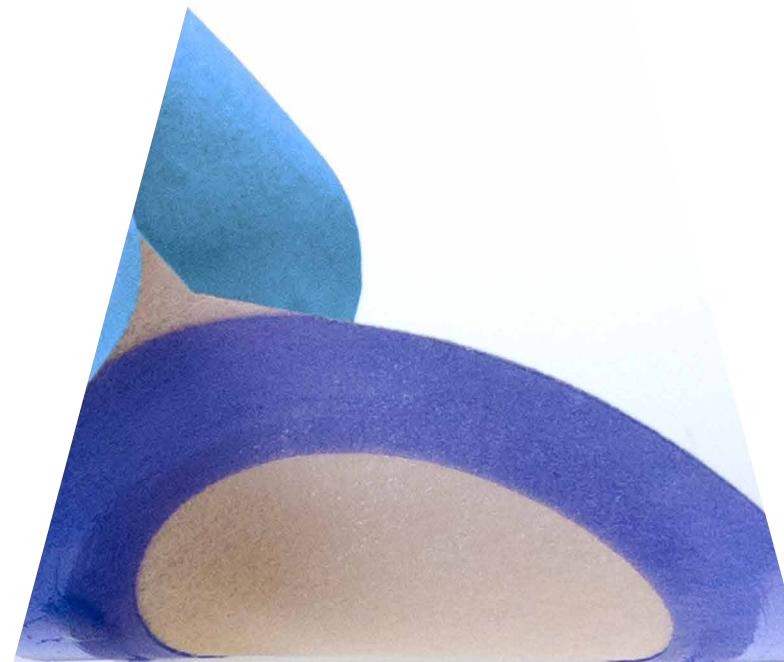
- Sterile 0.2µm hydrophobic barrier with a resealable gas-permeable membrane
- Fits all Ultra Yield Flasks as well as other brand flasks both glass and disposable
- AirOtop™ Enhanced Seals help improve microbial growth and are part of the Ultra Yield System
- Resealable for up to 24 hours in culture

Ultra Yield® Vented Screw Cap

Vented Screw Caps feature a durable non-autoclavable design for use on Thomson Ultra Yield® Flasks. They also include a sterile 0.2µm hydrophobic barrier for high-throughput gas exchange

Key Features

- Sterile 0.2µm hydrophobic barrier with a gas-permeable membrane
- Improve microbial growth



Disposable, Sterile, Easy to Use

Higher Gas Exchange on All Types of Shake Flasks

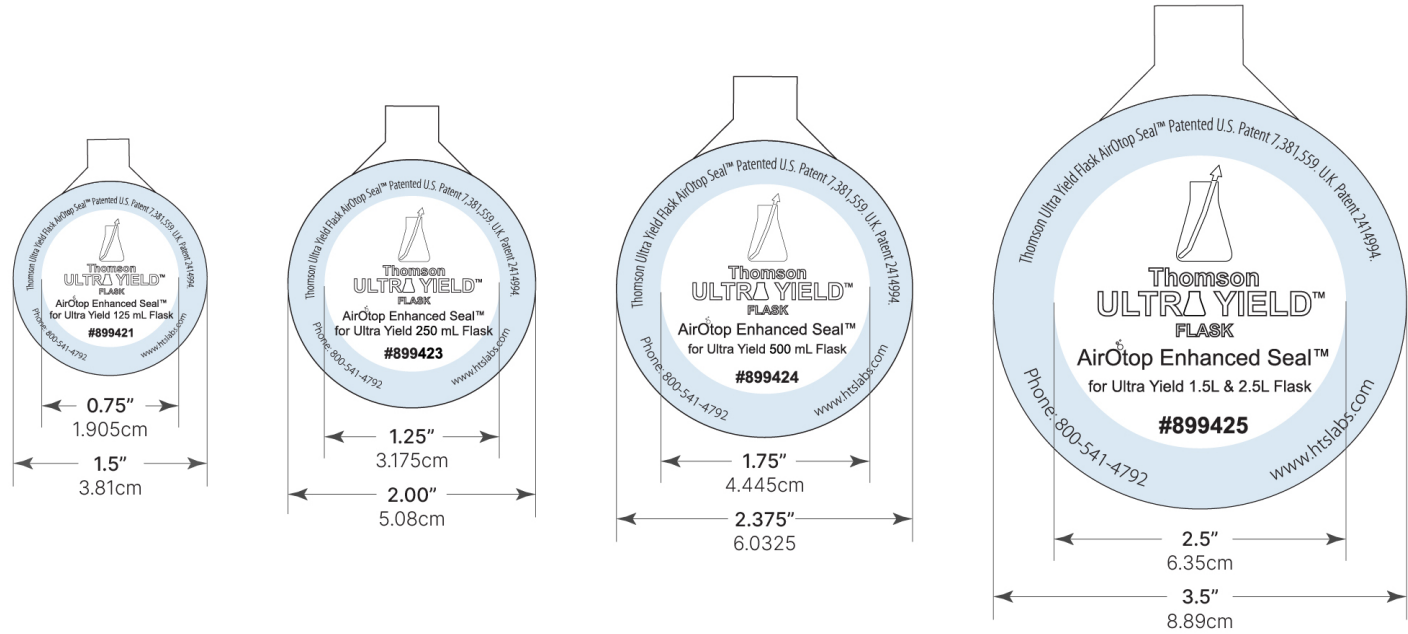
Sterile Membrane Barrier

AirOtop™ Seals and Vented Screw Caps feature a 0.2µm sterile membrane barrier which provides a higher gas exchange on all types of shake flasks. As a matter of fact, AirOtop™ Enhanced Seals are designed to fit on the tops of both Thomson flasks and traditional glass flasks. To determine the correct AirOtop™ Seal to use with your glass or disposable flask refer to AirOtop™ Enhanced Seal Compatibility below.

Resealable for Up to Approximately 24 Hours

AirOtop™ Seals are disposable, sterile, easy to use, and resealable for up to approximately 24 hours. Multiple sizes are available to keep all of your flasks covered. Testing has been conducted at multiple customer sites with great results. Organisms tested include Protista (Algae), *E. coli*, and other microbes which exhibited increased cell density, a more neutral media pH and increased gas exchange rates.

AirOtop Enhanced Seals Are Fully Compatible With Other Manufacturer's Flasks



Thomson AirOtop™ Enhanced Seals are fully compatible with other manufacturer's flasks.

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			★



Time to Harvest?

Ultra Yield Bidirectional Transfer Caps

Aseptic Transfer System

When it comes time to harvest flask contents or transfer to larger fermenters Thomson manufactures a single-use Ultra Yield Bidirectional Transfer Cap for the 1.5L and 2.5L Ultra Yield® Flasks. This aseptic transfer system keeps your process sterile and GMP-compliant



Applications & Protocols



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

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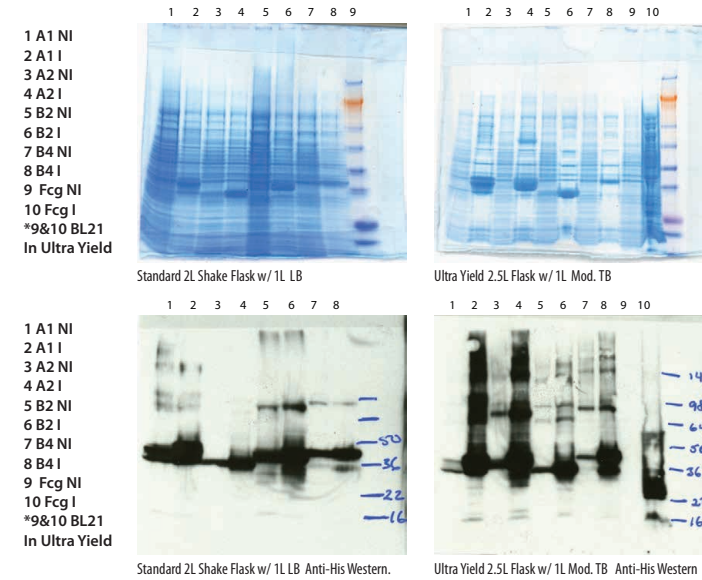
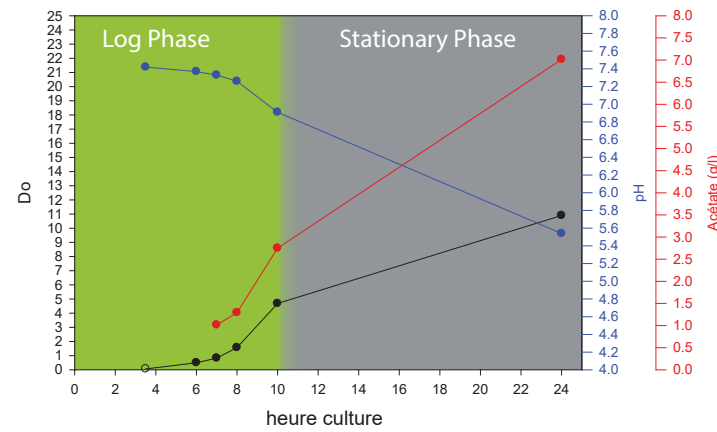
⁴ Pfizer, Inc., 10777 Science Center Drive, San Diego, CA 92122

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.

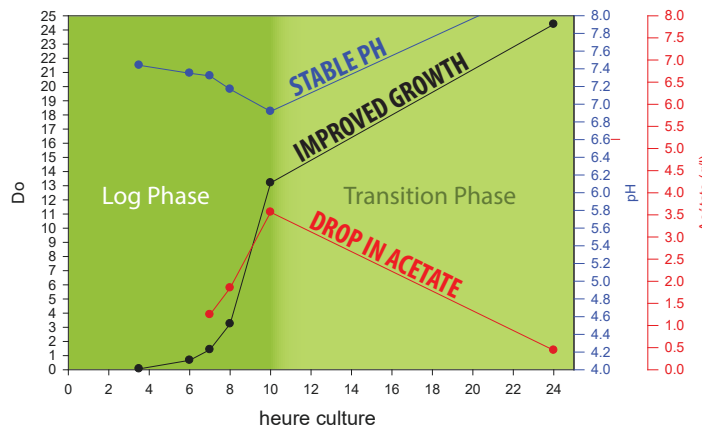
Conclusion

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]



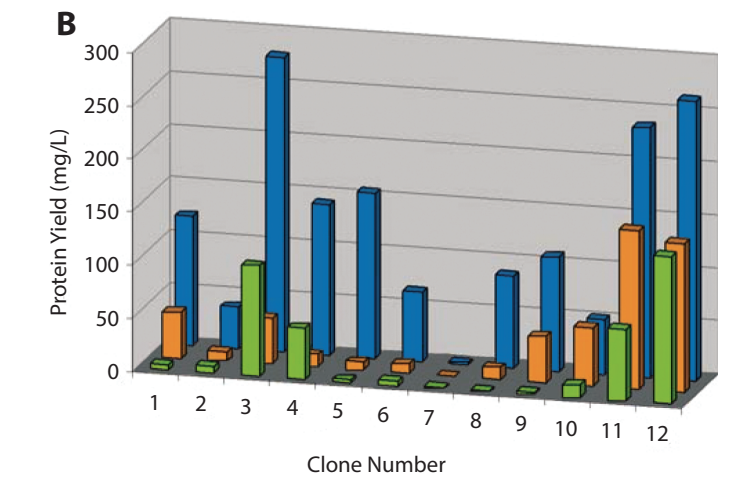
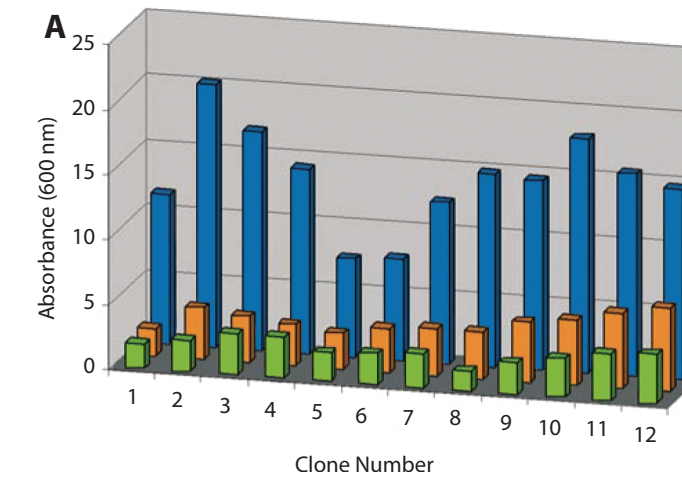
J. Struct. Funct. Genomics. 7, 101-8). These data demonstrate that the Ultra Yield[®] Flask substantially reduces the protein production footprint, and may make traditional fermentation approaches unnecessary at smaller scales.

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.



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

● O.D. ● pH ● Acetate g/L

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

Ultra Yield Flask

Standard Protein Protocol

Description

The 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.

Part Numbers

Ultra Yield® Flasks

Flask Size	125mL	250mL	500mL	1.5L	2.5L
Part #	931147	931144	931141	931138	931136-B
Seal Compatibility	AirOtop™ (899421)*	AirOtop™ (899423)*	AirOtop™ (899424)*	AirOtop™ (899425)*	AirOtop™ (899425)*
Flask Material	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)	PP (polypropylene)
Top Style	threaded	threaded	threaded	threaded	threaded
Top	sold separately	sold separately	sold separately	sold separately	sold separately
Working Volume	40 - 50mL	75 - 110mL	125 - 200mL	250-350mL	500mL - 1L
Shake Speed	300-350 RPM	300-350 RPM	300-350 RPM	300-350 RPM	300-350 RPM
Baffles	Yes	Yes	Yes	Yes	Yes
Sterility (SAL)	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶
Qty/Case	50	50	25	12	6

AirOtop™ Enhanced Seal

Flask Compatibility	125mL	250mL	500mL	1.5L & 2.5L
Part #	899421	899423	899424	899425
Membrane	Sterile 0.2µm hydrophobic barrier	Sterile 0.2µm hydrophobic barrier	Sterile 0.2µm hydrophobic barrier	Sterile 0.2µm hydrophobic barrier
Pore Size	0.2µm	0.2µm	0.2µm	0.2µm
Sterility (SAL)	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶
Qty/Case	100	100	100	100

Vent Cap

Flask Compatibility	125mL	250mL	500mL	1.5L & 2.5L
Part #	899109	899110	899111	899566
Membrane	PTFE	PTFE	PTFE	PTFE
Porre Size	0.2µm	0.2µm	0.2µm	0.2µm
Sterility (SAL)	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶	10 ⁻⁶
Qty/Case	50	50	25	24

Plasmid+® Media





Part #	446300
Final Product	Liquid Medium
Preparation Method	ready to use
Target Organism	<i>E. coli</i>
Size	1L
Sterility (SAL)	10 ⁻⁶
Qty/Case	6

Ultra Yield® Bidirectional Transfer Cap

Part #	899136
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)
Style	Threaded
Material	PP (polypropylene)
Sterility (SAL)	10 ⁻⁶
Air Filter Ventilation	0.2µm PTFE vent filter
Qty/Case	6

* Sold separately



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