THOMSON INSTRUMENT COMPANY Solutions™ At Work

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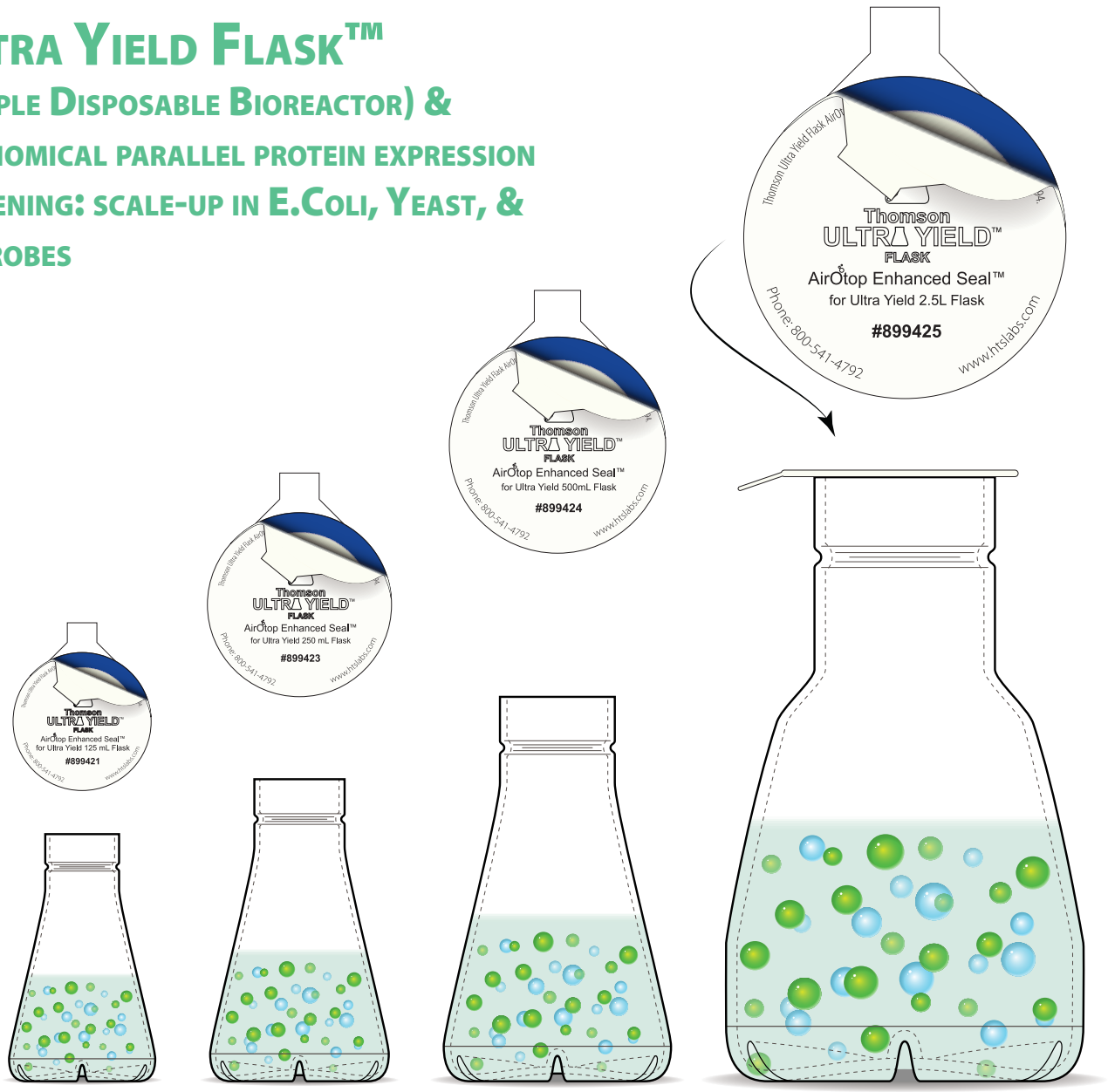
Ultra Yield Flask™

(Simple Disposable Bioreactor) & Economical parallel protein expression screening: scale-up in E.Coli, Yeast, & Microbes



125mL | *Part No. 931147*
Seal | *Part No. 899421*

250mL | *Part No. 931144*
Seal | *Part No. 899423*

500mL | *Part No. 931141*
Seal | *Part No. 899424*

2.5L | *Part No. 931136-B*
Seal | *Part No. 899425*

Pfizer · Genentech IN BUSINESS FOR LIFE · gsk GlaxoSmithKline

THOMSON INSTRUMENT COMPANY Solutions™ At Work

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

Introduction

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 anerobic 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):

Part #	Description	Media (mL)/Flask	Shaker Speed
931147 *Please cover the flask with Enhanced seal 899421.*	125mL Ultra Yield Flasks 50/case -- Sterile	40-50mL/flask	300-350 RPM
931144 *Please cover the flask with Enhanced seal 899423.*	250mL Ultra Yield Flasks 50/case -- Sterile	75-110mL/flask	300-350 RPM
931141 *Please cover the flask with Enhanced seal 899424.*	500mL Ultra Yield Flasks 50/case -- Sterile	125-200mL/flask	300-350 RPM
931136-B *Please cover the flask with Enhanced seal 899425.*	6/case 2.5L Ultra Yield Flasks -- Sterile	500mL (optimum)	300-400 RPM
931136-B *Please cover the flask with Enhanced seal 899425.*	6/case 2.5L (Fermentor Mode)-- Sterile	1L	250 RPM

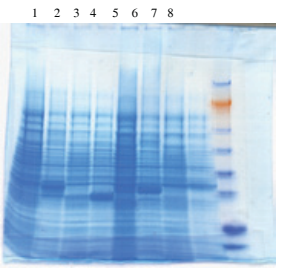
Results

Genentech, Inc

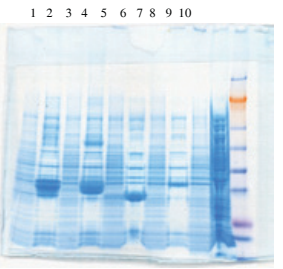
1 A1 NI
2 A1 I
3 A2 NI
4 A2 I
5 B2 NI
6 B2 I
7 B4 NI
8 B4 I

9 Fcg NI
10 Fcg I

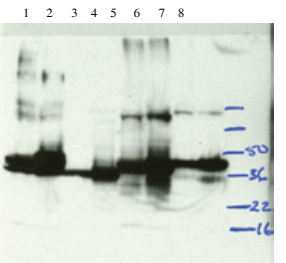
*9&10 BL21
In Ultra Yield



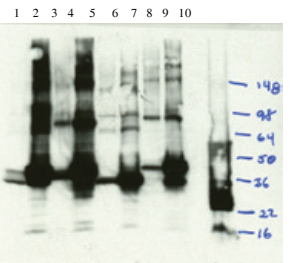
Standard 2L Shake Flask w/ 1L LB



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

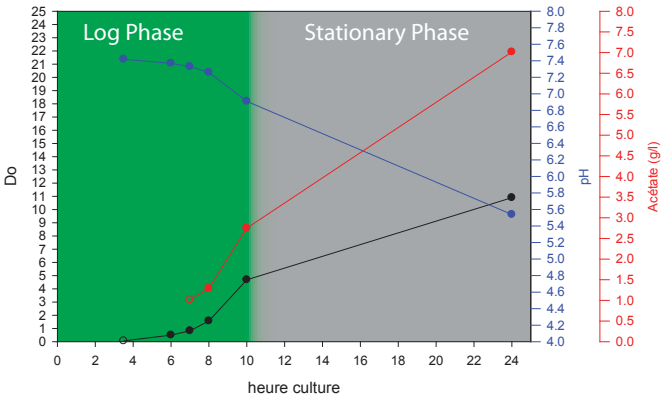


Standard 2L Shake Flask w/ 1L LB Anti-His Western.

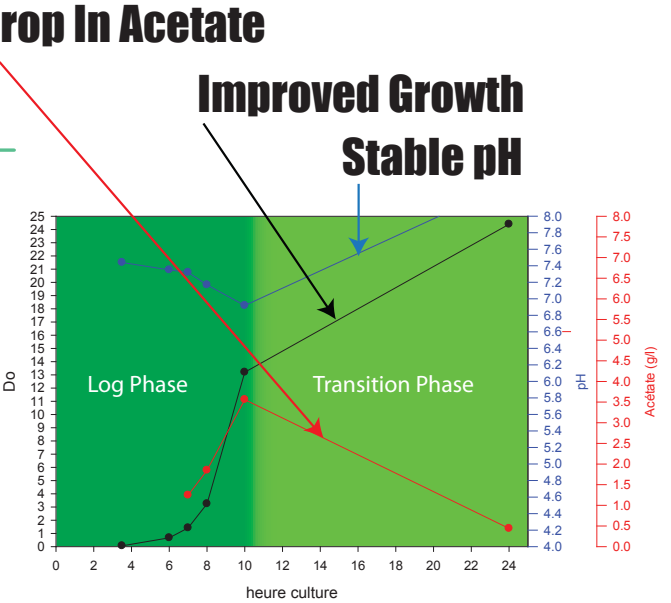


Ultra Yield 2.5L Flask w/ 1L Mod. TB Anti-His Western

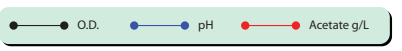
GlaxoSmithKline



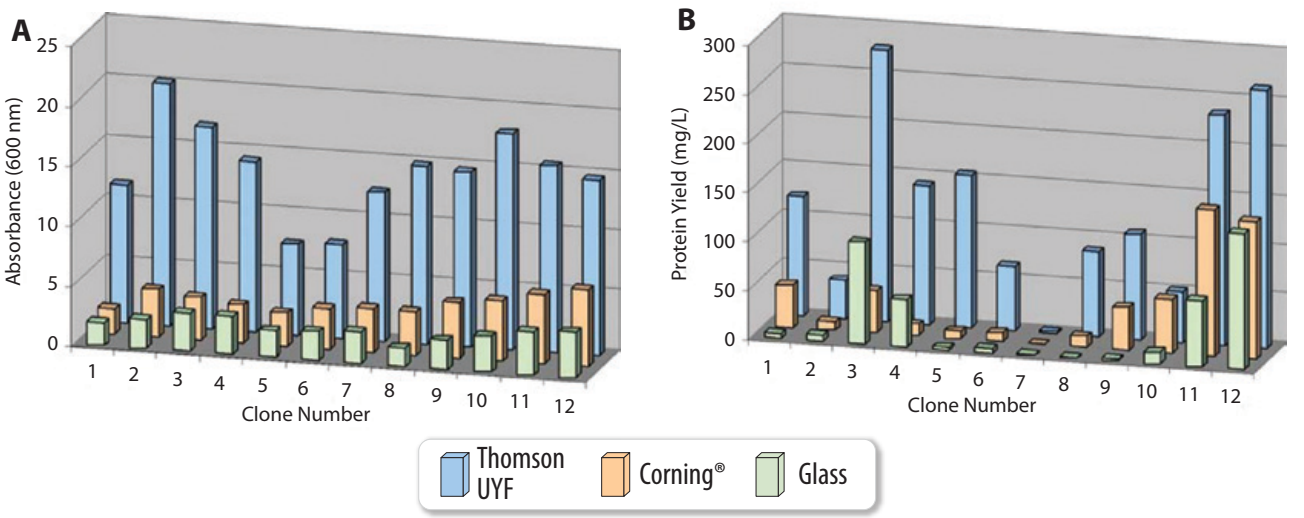
E.coli : Growth profiles **with classical glass flask** (2 L, no baffle), 400 mL Thomson medium, 400 rpm orbital shaker



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



Pfizer ULTRA YIELD™ 610% Yield Increase*



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 Genomics2006 Jun;7(2):101-8. Epub 2006 Dec 23.

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.

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+ or Protein Gold, and others are suitable examples of enriched media. The pH balance is most useful with buffers that contain phosphate since these are not temperature dependant. Personaly 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+ or Protein Gold.

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 20C, and up to 50h at 37C.

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, ATR and Kuhner.

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U.S. Patent 7,381,559. U.K. Patent 2414994.