

Ultra Yield™ Flask Standard Protein Protocol

Description

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.



Bacteria strains

E. coli DH5 α , 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 50 mL. Cultures less than 50 mL may be inoculated directly from a glycerol stock or plate.
- Next, use a standard 250mL overnight Ultra Yield Flask and Enhanced AirOtop Seal with a 50mL working volume which produces approximately 5 production flasks. The overnight culture should be grown for at least 16 hours at a temperature of 37deg C at 350rpm. This should be done in an orbital shaker using flask clamps.

Overnight Flask Production

Fill your production Ultra Yield 2.5L Flask to a working volume range of 500mL to 1L. This should consist of an enriched media like TB using a MOPS buffer at a pH of 7.4. The final optical density (OD @ 600nm) can be verified on a spectrophotometer. This will determine that the culture grew a high density.

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. Then transfer 5mL's of your overnight culture into the production Ultra Yield Flask using a pipette and seal the flask using a Thomson Enhanced AirOTop Seal. The flask can then be placed into an orbital shaker (using clamps) and set at a temperature of 37 ° C at (See Table 1) rpm. The cultures will be grown and expressed over a 24-hour period. Please note that 1:5000 of anti-foam should be used for limiting foaming of the culture. This is at the discretion of the end user.

Induction of the Ultra Yield Flasks (patented)

The cultures should be ready for induction after approximately 4 hours of agitation at 37 ° C or if the 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. Next, the temperature should be dropped to 18-25 ° C and left at an rpm of 300-350rpm for 500mL or 250-275rpm for 1L.

Harvesting of the Ultra Yield flasks (patented)

At the end of your 24 hour growth cycle remove the Ultra Yield Flasks from the shaker and take final measurements of your cultures for pH and OD(@ 600nm). Once this is done the pH can be adjusted to begin the purification process; by disruption, the cultures can be spun down or a lysis buffer added depending on the purification protocol.

Ultra Yield™ Flask Standard Protein Protocol (continued)

Recommended speeds (Table 1):

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 <i>Please cover the flask with Enhanced seal 899421.</i> | 125mL Ultra Yield Flasks 50/case -- Sterile | 40-50mL/flask | 300-350 RPM |
| 931144 <i>Please cover the flask with Enhanced seal 899423.</i> | 250mL Ultra Yield Flasks 50/case -- Sterile | 75-110mL/flask | 300-350 RPM |
| 931141 <i>Please cover the flask with Enhanced seal 899424.</i> | 500mL Ultra Yield Flasks 50/case -- Sterile | 125-200mL/flask | 300-350 RPM |
| 931136-B <i>Please cover the flask with Enhanced seal 899425.</i> | 6/case 2.5L Ultra Yield Flasks -- Sterile | 500mL (optimum) | 300-400 RPM |
| 931136-B <i>Please cover the flask with Enhanced seal 899425.</i> | 6/case 2.5L (Fermentor Mode)-- Sterile | 1L | 250 RPM |

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 48 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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