

Application Note:

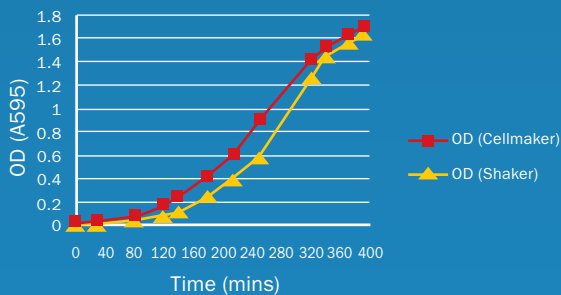
Seamless scale-up of E.coli shaker flask culture to 8L and 50L production in the CellMaker Regular single-use bioreactor system.

QUALITY | RELIABILITY | SERVICE EXCELLENCE | PRODUCTIVITY

Introduction

E. coli is still the system of choice for recombinant protein expression in many cases. The quick and relatively inexpensive procedures involved potentially provide the most efficient route to good yields of recombinant protein. The CellMaker Regular can seamlessly enable scale-up from shaker flask to 8L and 50L single vessel production. Exceptionally fast setup is possible with the Cellexus CellMaker Regular single-use, disposable bioreactor; prewarmed media (+ antibiotics and antifoam) is delivered to the Cellexus CellexusBag via a peristaltic pump and the fermentation is ready to be initiated by addition of inoculum (eg. 1/200 dilution of overnight culture). The CellMaker Regular produces a similar growth profile to a conventional (baffled) shaker flask initiated in parallel (50ml culture volume in 250ml flask). Higher ODs can be achieved in the CellMaker Regular by using richer media (e.g. Terrific broth) or incorporating a feeding strategy.

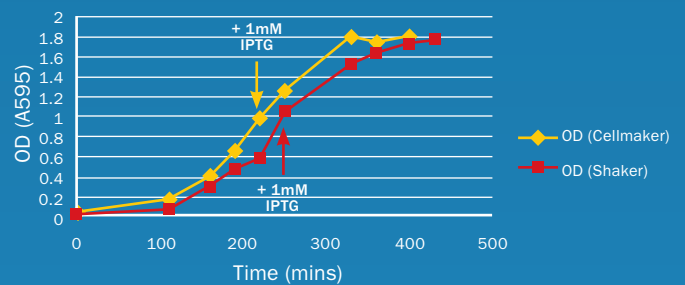
LB Growth Curve @ 37°C



Standard recombinant protein expression induction (e.g. from the T7 promoter following induction of T7 RNA polymerase (via the lacUV5 promoter) by IPTG) can readily be transferred to 8L or 50L scale with the Cellexus CellMaker Regular. Routinely, short inductions (3-4hrs) are used for protein expression at 37°C or longer inductions for expression at lower temperatures (e.g. 20°C

overnight). The CellMaker Regular has the ability to cool as well as heat the culture media allowing single vessel low temperature inductions to be conducted. Low temperature expression inductions may be beneficial for poorly/partially soluble target proteins or proteins that are toxic to the host. The enhanced culture aeration achieved by sparging with an oxygen-enriched air supply, by virtue of the built-in oxygen generator, can also result in increased target protein yield and protein solubility.

LB Protein Induction (LP:65KDa)



Experimental details

Typical CellMaker Regular run parameters are shown in the table (below) for a 3hr induction of a recombinant protein at 37°C. Protein expression was induced by the addition of IPTG (to 1mM final concentration) once the culture had attained an OD of 1 (A595nm). Prior to induction the sparge gas supply (4L/min air) was supplemented with oxygen (4L/min), and the vent flow rate increased to maintain an overall bag pressure of 50mBar.

Following harvesting of the expression culture, samples were processed and subjected to whole cell protein analysis by SDS-PAGE. Expression profiles of three distinct recombinant proteins are shown in the panel (over) (target protein indicated by arrow (C=CellMaker, S=Shaker, U=Uninduced)).

TIME (mins)	OD [Shaker]	Set Temp	Process Temp	Gas Mix (Air/O2/Headspace) (all L/min)	Pressure (mBar)	Comments
0	0.039 [0.042]	37	32.1	4/0/0.4	40	
110	0.162 [0.097]	37	37	4/0/0.4	50	
160	0.390 [0.314]	37	37	4/0/0.4	50	Input O2
190	0.656 [0.489]	37	36.8	4/0/0.4	50	
220	0.986 [0.589]	37	37	4/0/0.4	50	+ 1mM IPTG Cellmaker
250	1.255 [1.062]	37	37	4/0/0.4	50	+ 1mM IPTG Shaker
330	1.794 [1.529]	37	37	4/0/0.4	50	
360	1.768 [1.642]	37	36.8	4/0/0.4	50	
400	1.787	37	37	4/0/0.4	50	Harvest Cellmaker
430	[1.772]					Harvest Shaker

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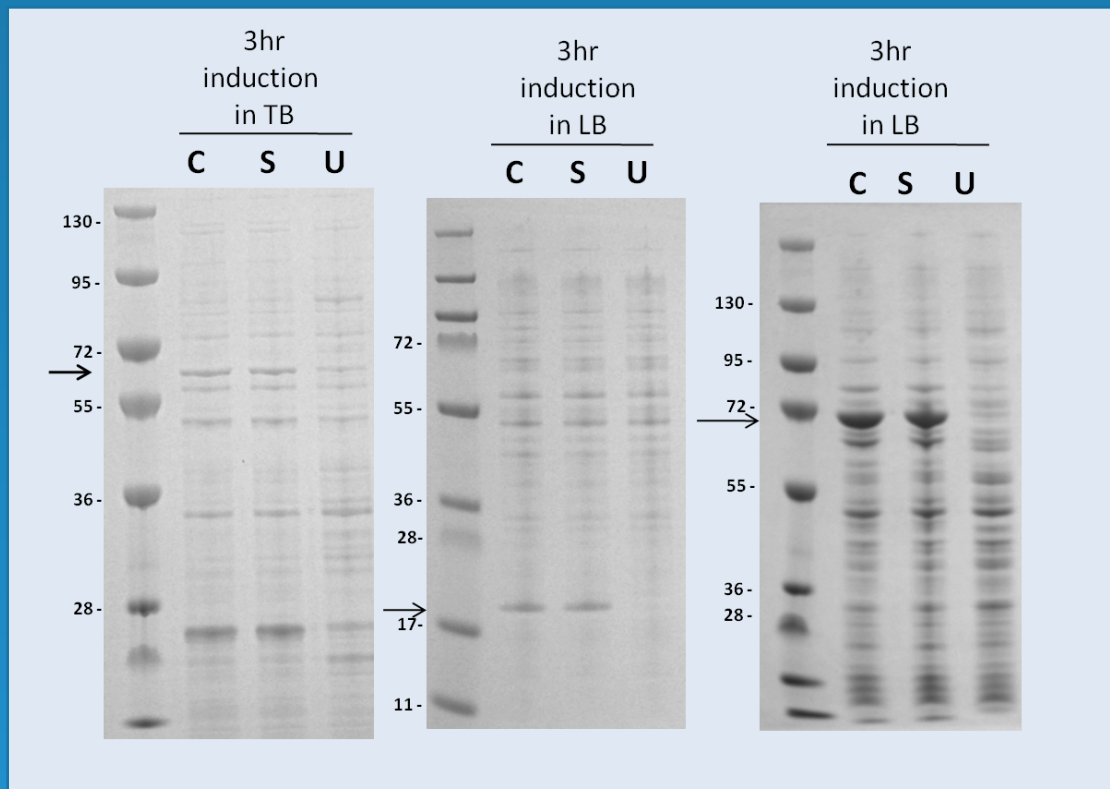
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Conclusions

This data demonstrates seamless scale-up from shaker flask culture to 8L and 50L production in the CellMaker Regular system. Further optimization of run parameters may also offer the benefit of increased yield of target protein above those achievable in shaker flasks.

The Cellexus CellMaker Regular offers an exceptionally efficient platform for scale-up of E.coli culture. With rapid set-up times, the disposable format eliminates cleaning and contamination issues, and the stand alone single vessel system overcomes issues with multiple flasks/ incubators.



For further details, or to request a quotation, contact us now.

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