

May 27, 2025

Master_Setup and Operation of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD).

DOI

<https://dx.doi.org/10.17504/protocols.io.n92ld897ov5b/v1>

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Protocol Citation: Nathan Wright 2025. Master_Setup and Operation of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD).. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.n92ld897ov5b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: April 23, 2024

Last Modified: May 27, 2025

Protocol Integer ID: 98783

Keywords: Single-Use, Bubble Column Reactor, e.coli, protein production, SBDD, crystallising disease target protein, based drug discovery, protein crystallography, flask workflows for protein expression, drug discovery, approach to drug design, drug design, medicines discovery, multiple protein, centre for medicines discovery, molecule binder, disease target protein, protein quantity, structural biology, scale expression of recombinant protein, protein, column bioreactor, protein expression, bioreactor array, high demand for protein quantity, obtaining sufficient protein quantity, increasing protein production efficiency, protein production efficiency, crystal form, recombinant protein, crystallisation, sufficient protein quantity, crystallisation behaviour

Funders Acknowledgements:

National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)

Grant ID: U19AI171399

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Abstract

Structure-Based Drug Discovery (SBDD) is an approach to drug design which involves crystallising disease target proteins unbound and with candidate small-molecule binders, to observe and analyse the binding interaction, or to identify novel binders that can be developed into lead-like compounds.

Obtaining sufficient protein quantity and crystal forms amenable to soaking with candidate binders remains a bottleneck. The Centre for Medicines Discovery's (CMD) approach to this problem, is to design multiple constructs to explore protein expression and crystallisation behaviour. Protein crystallography (PX) has a high demand for protein quantities, a limiting factor in this workflow is the production of sufficient quantities of the multiple proteins for our experimental needs. This burden increases rapidly with the addition of each construct.

Existing shake-flask workflows for protein expression do not scale adequately to meet these requirements. To address this bottleneck a novel, single-use Bubble-Column Bioreactor (suBCR) array was developed, that allows for parallel 1 L *E. coli* batch cultivation. This bioreactor array will be incorporated into a Gene-to-Product workflow aimed at increasing protein production efficiency by increasing throughput whilst reducing labour burden.

Materials

Media (auto-induction):

Formedium AIMTB0310 <https://formedium.com/product/aim-terrific-broth-base-including-trace-elements/>
Ni-NTA Magnetic Beads (42179.02) from Serva (<https://www.serva.de/>)

Lysis reagent: FastBreak (V857C or V8571) from Promega (<https://www.promega.co.uk/>)

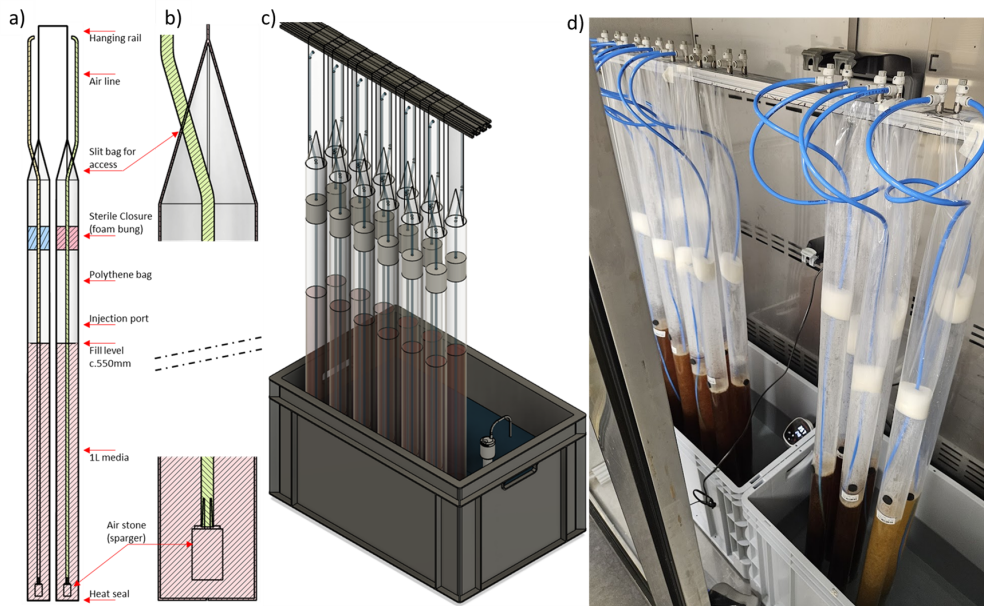
Before start

The reader should be familiar with the companion protocol: "The Manufacture and Setup of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD)."

Culture Main Bags

4h 32m

1



Bag setup: a) Guidance for Bag manufacture and assembly, b) Arrangement of the Bags over the hanging rail, c) A Bag Array in operation.



Bag set-up a) Bags removed from water bath for illustrative purposes, b) a typical bubble swarm and foam layer, c) close-up of the Bags in the water bath with heaters, d) a heat map showing water bath heating of the Bags.



with heaters, or a heat map showing water bath heating of the bags, left-to-right Bags acclimating.

2 Prepare and autoclaved Terrific Broth Base including Trace elements (Formedium AIMTB310)

2.1 To each litre of sterilised media:

Add  1 mL 10% Antifoam 204

Add  1 mL 1000x antibiotic stock (e.g. Kanamycin 50mg/ml)

Add  20 g glycerol

3 Equilibrate the incubator bath:

3.1 Start the immersion heater program  37 °C  06:00:00

3.2 Allow incubator to reach set temperature.



4 Loading and equilibrating the BCR bags:

4.1 Add  1 L prepared AIM TB media to each BCR bag.

4.2





The bag with airline inserted, and a funnel being used to demonstrate the filling process.

4.3 Transfer BCR bags to incubator.

4.4 Insert sparger stones into BCR bag, making sure they are at the bottom of the BCR.



4.5 Attach sparger stones to air control valves. Set air flow, 1 lpm.

4.6 Allow bags to equilibrate.

5 Inoculate Media:

5.1

Note

If cultures are inoculated by late morning biomass growth can be monitored in person. If choosing to manually induce protein expression by IPTG this can be done before the end of the work day.

Expression can run unattended overnight.

5.2 Add  10 mL starter culture, per L main culture.

6 Biomass Growth Phase:

6.1 Continue incubation  37 °C ,  04:00:00 (approximately).

4h



Note

Incubation at  37 °C should continue until the culture has entered the exponential growth phase. Typically this takes 4-6 hours given the conditions described. Autoinduction media will trigger protein expression once all the glucose in the media has been consumed, this happens at OD600 of between 3-5 in most cases. The incubation temperature should be reduced before the autoinduction point to promote protein solubility.

7 Protein Expression Phase:

7.1 Incubate (ambient)  Overnight (approximately 16 h)

- [EndPoint] $\Delta OD_{600} \approx 0$

Note

Without external cooling cultures' metabolic activity will heat the culture to  25-27 °C . If working with proteins with temperature sensitive expression, an external recirculating chiller can be connected to the water bath.

7.2

Note

- A typical culture, having been inoculated at between 10-12 pm on day 1, can be harvested in the morning of day 2. This gives a total culture time of 20-24 hours.
- Culture times of up to 36 hours have been tried successfully, but this is likely to be cell/protein specific.
- Short expression experiments can be performed with a c.3 hour induction period following the addition of IPTG.
- Culture biomass can be measured by taking Optical Density readings at 600 nm in a spectrophotometer. The relationship between OD600 and biomass is non-linear at high concentrations. If using a fixed path-length or cuvette, dilute samples such that readings remain below 1 OD unit.
- If using autoinduction media and cell growth appears to have stalled below OD600=5, IPTG can be added to ensure induction has occurred.

7.3

**Note**

- In rich media biomass will continue to increase after induction.
- Once OD600 values plateau late log phase has occurred, and cell harvest must occur before OD600 begins to drop when death phase begins.

8 Harvest:

8.1 Take final OD600 reading

8.2 Collect  1 mL culture samples for diagnostic testing

8.3 Transfer culture to 1 L centrifuge bottles

Note

The Bags are long and flexible, decanting by pouring culture out from the top of bags can lead to spillage.

Place Bags into a large jug or similar. With a sharp implement, make a small incision in the bag and allow it to drain into the jug.

There is a lot of hydrostatic pressure on the liquid at the bottom of the Bags when full. To avoid splashing make incisions towards the top of the liquid level at first. As the culture drains, larger incisions can be made at the bottom to drain all liquid and sediments.

8.4 Pellet the cell,  4000 rcf, 4°C, 00:30:00

30m

8.5 Drain spent media and sterilise with 1 tablet/L Virkon

8.6 Transfer cell pellet to cryo-safe sample bags. Mark with full and clear tracking information.



8.7 Record wet cell weight (g)

8.8 Flash freeze the cell pellet in liquid nitrogen.

8.9 Store cell pellet  -80 °C

9 Overexpression QC:

9.1

Note

Using Nickel Magnetic Beads perform a rapid over-expression assay. The protocol described here is abridged and achieves only incomplete cell lysis, but can be used to verify over-expression in 5-10 minutes, plus gel running time.

9.2 Transfer 1 ml of culture sample to a microcentrifuge tube  [go to step #8.2](#)

9.3 Add  100 µL lysis reagent to the sample

Note

If biomass is low or protein expression levels are expected to be low, take a larger culture sample and pellet it first. This will increase the target protein yield but you may need to check sufficient lysis has occurred.

9.4 Incubate  Room temperature  00:02:00 in a tube rotor

9.5 Pellet the cell debris at maximum speed in a microtube centrifuge
 16000 rpm, 00:02:00

**Note**

There is no need to spin at  4 °C unless you plan to use the eluted protein for other purposes than QC SDS-PAGE.

9.6 Aspirate soluble fraction (**mixed proteins**) with pipette

9.7 Add  50 µL magnetic IMAC beads to the soluble fraction (**mixed proteins**)

9.8 Incubate  Room temperature  00:05:00 in a tube rotor

9.9 Incubate  Room temperature  00:01:00 in a magnetic separator stand

9.10 Aspirate soluble fraction (**unbound material**), reserve for SDS-PAGE analysis.

9.11 Wash the retained beads  2 mL binding buffer  00:01:00

9.12 Incubate  Room temperature  00:01:00 in a magnetic separator stand

9.13 Aspirate soluble fraction (**unbound material**), reserve for SDS-PAGE analysis.

9.14 Add  50 µL elution buffer to the mag beads

2m

9.15 Incubate  00:02:00  Room temperature

9.16 Incubate  00:01:00 in a magnetic separator stand



- 9.17 Aspirate soluble fraction (**eluted protein**) with pipette
- 9.18 Measure the A280 using a spectrophotometer (e.g. NanoDrop)
- 9.19 Run an SDS-PAGE gel, to check for over-expression and quality control

Results:

- 10 Tracking IDs and Yields:

Expected result

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//Comments
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[illegible]



11 Annotated SDS-PAGE gel of Mag Prep test purifications:

Expected result