



Jul 29, 2025

🌐 Setup and Operation of Single Use (SUB) Bubble Column Reactors (BCR) for Recombinant Proteins: Litre-Scale Expression of enterovirus 3C protease for Structural Biology and Drug Design (SBDD)

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgqp4ykvk5/v1>

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Protocol Citation: Nathan Wright 2025. Setup and Operation of Single Use (SUB) Bubble Column Reactors (BCR) for Recombinant Proteins: Litre-Scale Expression of enterovirus 3C protease for Structural Biology and Drug Design (SBDD). protocols.io <https://dx.doi.org/10.17504/protocols.io.36wgqp4ykvk5/v1>

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Protocol status: Working

We use this protocol and it's working

Created: July 25, 2025

Last Modified: July 29, 2025

Protocol Integer ID: 223266

Keywords: Single-Use, Bubble Column Reactor, 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, 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, E. coli, EV-D68, EV-A71, 3C protease, scale expression of enterovirus 3c protease, enterovirus 3c protease, recombinant expression of enterovirus 3c protease, parallel processing of multiple 3c protease variant, multiple 3c protease variant, using magnetic bead purification, a71 3c protease, 3c protease, use bubble

Funders Acknowledgements:

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

Grant ID: U19AI171399

Disclaimer

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Abstract

Recombinant expression of enterovirus 3C proteases from EV-D68 and EV-A71 strains requires scalable cultivation methods to produce sufficient protein quantities for structural biology applications. This protocol describes the setup and operation of single-use Bubble Column Reactors (suBCR) for parallel 1L E. coli batch cultivation of 3C protease constructs. The system uses auto-induction Terrific Broth media with controlled aeration and temperature management, transitioning from 37°C growth phase to ambient temperature for overnight protein expression.

The protocol successfully produced high-density cultures with final OD₆₀₀ values of 12.6 for EV-D68 3C protease and 17.6 for EV-A71 3C protease, yielding 244.6g and 202.1g wet cell weight respectively from individual 1L cultures. Quality control using magnetic bead purification and SDS-PAGE analysis confirmed successful overexpression of both constructs. The suBCR array enables parallel processing of multiple 3C protease variants while reducing labor requirements compared to traditional shake-flask methods, with total culture times of 20-24 hours from inoculation to harvest.

Attachments



[PAGE25-00819.pdf](#)

3.6MB



[PAGE25-00822.pdf](#)

1.8MB

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/>)

Protocol materials

⊗ EV-D68 3C protease; aliases: Enterovirus A71 3C protease, non cleavable C-term 6xHis tag **addgene Catalog #204817**

⊗ Enterovirus A71 3C protease **addgene Catalog #204816**

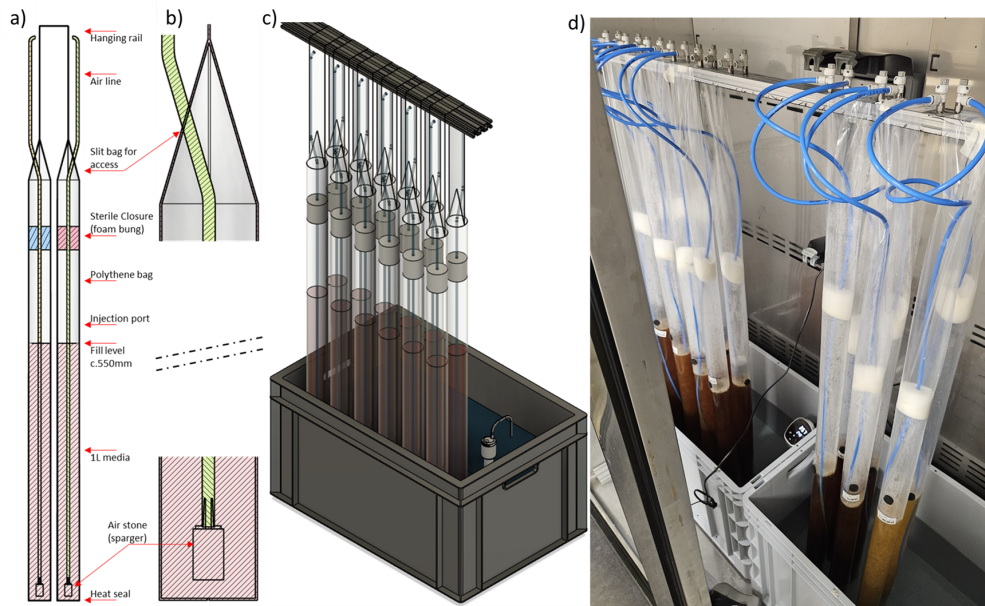
Before start

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 For protein expression we used the following plasmids:



EV-D68 3C protease; aliases: Enterovirus A71 3C protease, non cleavable C-term 6xHis tag **addgene Catalog #204817**



Enterovirus A71 3C protease **addgene Catalog #204816**

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:

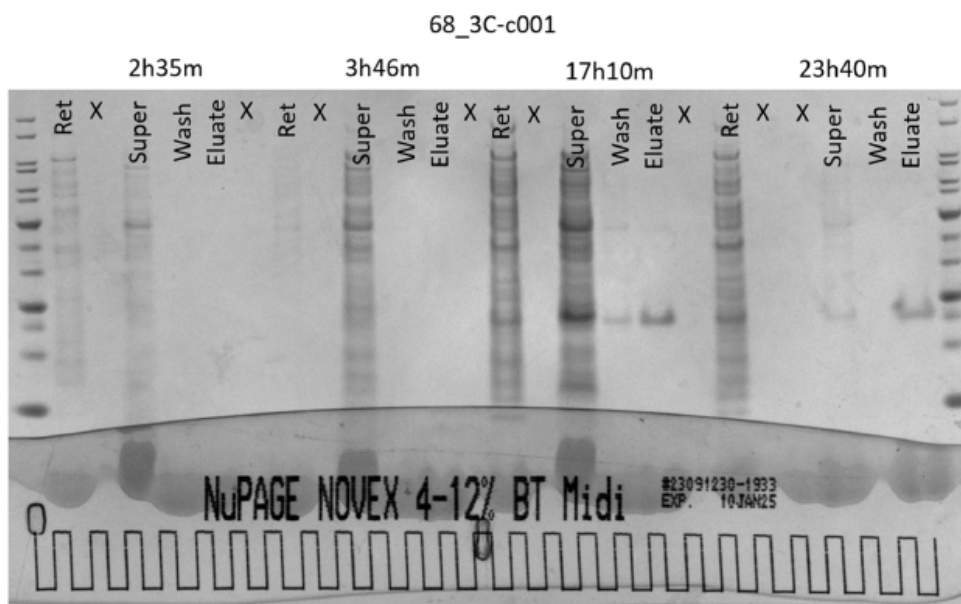
10 Tracking IDs and Yields:

	A	B	C	D	E	F	G	H	I	J
	ELN title	Target ID	Clone ID	Construct ID	Expression ID	Culture Total Volume (L)	OD600	Pellet wet cell weight (g)	SDS-Page : Test purification mag prep (pass)	Expression growth (pass)
	PAGE25-00819	D68E V3CP ROA	D68E V3CP ROA-k001	D68EV3CPROA-c001	D68EV3CPROA-e006	10	12.6	244.6	Pass	Pass
	PAGE25-00822	A71E V3CP ROA	A71E V3CP ROA-k001	A71EV3CPROA-c001	A71EV3CPROA-e005	9	17.6	202.1	Pass	Pass

Ids for internal reference and yields achieved for both 3C protease constructs used in this protocol.

11 Annotated SDS-PAGE gel of Mag Prep test purifications for 3C protease:

Expected result

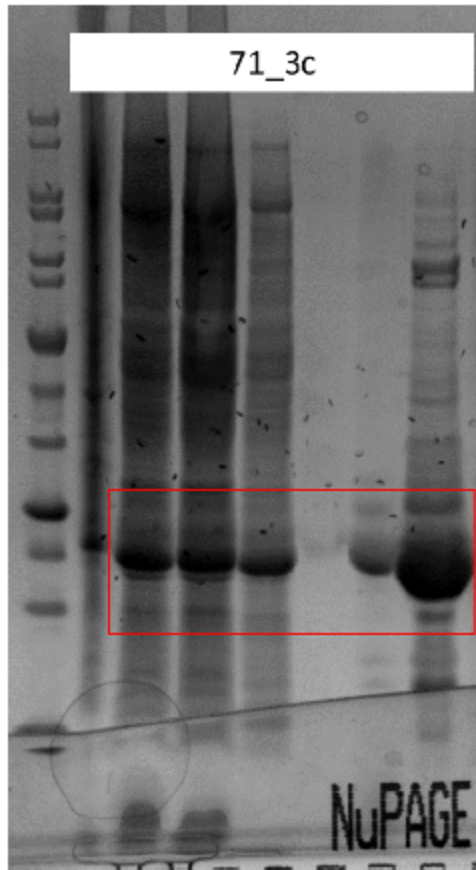


SDS-PAGE EV-D68 3C protease

12 Results obtained for 3C protease EV-A71.

Expected result

230912301932annotated



SDS-PAGE Analysis of EV-A71 3C Protease Expression and Magnetic Bead Purification