

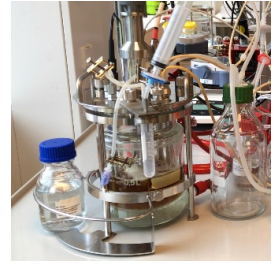
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## Protein expression in *Bacillus subtilis*

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**Protocol status:** Working

**We use this protocol routinely and it works for us**

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**Keywords:** *B. subtilis*, Bacillus, Bacillus subtilis, Protein expression, bacterial glutamic peptidase, protein expression, secretion of polypeptide, positive bacteria, protein production, protein, proteins into the extracellular medium, polypeptide, other strain, excellent fermentation property

## Abstract

*B. subtilis* is a gram-positive bacteria used by both academia and industry as a protein production workhorse. This is due to its' their excellent fermentation properties, high production titers, and capacity to secrete proteins into the extracellular medium.

This protocol describes how to express proteins in *B. subtilis*. The protocol is developed using KO7-S, although it might also work for other strains as well. The method is adapted from Rasmussen, M. D.; Bjoernvad, M. E.; Diers, I. Pectate Lyase Fusion for Expression and Secretion of Polypeptides. WO 00/75344, 2000 and Jensen, K.; Østergaard, P. R.; Wilting, R.; Lassen, S. F. Identification and Characterization of a Bacterial Glutamic Peptidase. *BMC Biochem.* **2010**, 11 (1), 47. <https://doi.org/10.1186/1471-2091-11-47>.

## Materials

### MATERIALS

☒ Sodium molybdate dihydrate

☒ manganese sulfate

☒ Iron(III) chloride hexahydrate **Catalog #236489**

☒ Zinc sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #204986**

☒ Magnesium sulfate heptahydrate

☒ Sodium Phosphate dibasic **Fisher Scientific Catalog #S373-500**

☒ Copper (II) sulfate pentahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #209198**

☒ Yeast extract

☒ Nalgene®; Rapid-Flow®; Sterile Disposable Bottle Top Filters with PES Membrane, 150mL, 0.45µm pore, 45mm neck **Thermo Fisher Catalog #296-4545**

☒ Maltodextrin (DE 13.0-17.0) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #419680**

☒ Pluronic L-61 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #435422**

## Troubleshooting

## Safety warnings

 Be sure to wear protective equipment when adjusting the pH of the media. Follow local safety regulations

## Before start


Make sure you have your expression strain freshly streaked on an agar plate.

## Cal18-2 media preparations



- 1 Prepare a stock solution of 2.0g/L  $\text{Na}_2\text{MoO}_4$ . Sterilize by filtration
- 2 Prepare a trace metal solution consisting of
  - 4.48g/L  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$
  - 3.33g/L  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
  - 0.625g/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
  - 7.12g/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$Sterilize by filtration
- 3 Fill a blue cap bottle to ~80% of the desired final volume with MQ water.
- 4 Add a magnetic stirrer to the blue cap bottle and place the bottle on a stirring plate. Turn on the stirring, and make sure it's mixing well.
- 5 Add the following to the bluecap bottle:
  - 40g/L yeast extract
  - 1.3 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
  - 50 g/L maltodextrin (DE ~ 12)
  - 20 g/L  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$
  - 6.7mL/L 2.0g/L  $\text{Na}_2\text{MoO}_4$  stock solution
  - 6.7mL/L Trace metal solution
  - 100 $\mu\text{L}$ /L Pluronic L-61
- 6 Make sure that all of the ingredients are dissolved
- 7 Adjust to pH 6 with 5M NaOH
- 8 Add MQ water to the desired final volume
- 9 Sterilize by filtration

**Note**

The media easily clogs filters, so choose a 0.45µM vacuum bottle top filter for this step and be prepared to use a few filters per liter


- 10 Store the media at  4 °C until needed

**Overnight culture - Day 1**

- 11 Inoculate between  3 mL to  50 mL LB media with a single colony of the expression strain. Depending on the expression volume and overnight OD. The culture can be grown in in a 24-deepwell plate, a falcon tube or a shake flask

- 12 Grow the strain at  37 °C  Overnight

**Note**

Make sure to not incubate the overnight culture for longer than  16:00:00 . Using an overnight culture that has been incubating for longer than this, often results in non-reproducible results

**Expression - Day 2 - 4/5**

- 13 Prepare the desired volume of expression media in the desired vessel

**Note**

Vessel and media volume has a massive influence on the final protein titers. In our experience, 24-deepwell plates in an incubator with a large shaking amplitude and CSTRs work well, while shake flasks give low protein yields. This is likely highly dependant on the equipment (e.g. incubators and the shape and baffle pattern of the shake flasks), and thus should be optimized for the individual labs

- 14 Inoculate the expression media to an OD<sub>600</sub> of 0.1



- 15 Incubate the expression culture at 20 °C with 250 RPM shaking between 48:00:00 and 72:00:00

#### Note

The expression temperature and duration is dependant on the target protein, although the specified values seem to be a good starting point in our experience

## Harvesting - Day 4/5

- 16 Harvest the culture by centrifuging at 6000 x g, 4°C, 00:05:00

#### Note

Depending on what the samples are for, the supernatant from the first centrifugation can additionally be centrifuged at 16000 x g, 4°C, 00:30:00 to clear out cell debris and other smaller contaminants

- 17 Keep the sample On ice when working with it and at -20 °C for storage