# Isolation of the Secretome from *Bacillus subtilis*

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**[Abstract]** Bacteria are commonly known to secret proteins in large amounts into the surrounding environment in high concentrations via various pathways. These proteins can be involved in numerous processes like cell-cell communication, exopolymer formation but also metabolic active enzymes are secreted that are interesting for industrial production of proteins. One of the most regularly used organisms for industrial protein production is the Gram-positive bacterium *Bacillus subtilis* (*B. subtilis*). Here we describe a protocol that can be used to quantitatively and qualitatively analyze secreted proteins from *B. subtilis.*

## Materials and Reagents

1. *B. subtilis*
2. Trichloroacetic acid (Merck KGaA, catalog number: 1.00.807.0250)
3. Acetone (J.T.Baker®, catalog number: 9006-01)
4. BCA-assay (Pierce Antibodies, catalog number: 23225)
5. Casein Hydrolysate (Oxoid Limited, catalog number: LP0041)
6. (CH)-medium (see Recipes)
7. Solution G (see Recipes)
8. Resuspension Buffer (see Recipes)

## Equipment

1. 50 ml flasks with baffles
2. Acrodisc® syringe filters with a pore size of 0.2 µm (Pall, catalog number: 4652)
3. Refrigerated centrifuge (*e.g.* Eppendorf, model: 5810R)

## Procedure

1. Inoculate *B. subtilis* to an $OD_{600}$ of 0.1 in appropriate medium [*e.g.* CH, lysogeny broth (LB), Spizizen minimal *medium* (SMM); here we used 10 ml cultures in CH-medium].

 

2. Grow cells to respective $OD_{600}$ (here we used an $OD_{600}$ of 4; cells growing at 37 °C; 150 rpm in 50 ml flasks with baffles).
3. Harvest cells by centrifugation (≥ 2,800 *x g*; 4 °C; 20 min).
   *Note: Keep cell pellet as a control for later SDS-PAGE analysis.*
4. Transfer the supernatant into fresh tubes and centrifuge again (2,800 *x g*; 4 °C; 20 min).
5. Repeat step 4.
6. Collect the supernatant and sterile filter it using Acrodisc® syringe filters with a pore size of 0.2 µm.
7. Add ice cold trichloroacetic acid [90% (w/v) stock] to the solution to a final concentration of 10% (v/v). This will result in precipitation of the proteins in the solution.
8. Harvest the precipitate by centrifugation (11,000 *x g*; 4 °C, 5 min). A small white pellet should be visible.
9. Wash the pellet twice with 2 ml of ice cold acetone (PA grade or higher quality) and centrifugation (11,000 *x g*; 4 °C, 5 min).
   a. For quantitative analysis dry the pellet for 15 min at 60 °C to remove remaining traces of acetone. Furthermore, the pellet should be resuspended in $H_2O$ instead of buffer (step 10).
10. Resuspend the pellet in resuspension buffer (we used a volume of 50 µl).
    *Note: The volume can be scaled up or down with respect to your amount of cells (secreted proteins).*
11. The amount of secreted proteins can be analyzed by BCA-assay or analyzed by SDS-PAGE.
    *Note: As a control if the isolation has worked successfully the isolated secreted proteins and the pelleted cells (see step 3) should be resuspended in $H_2O$ or buffer (5-10x volume of pelleted cells) and analyzed by SDS-PAGE. The band pattern of the isolated proteins and the cells should be different. If the band pattern is similar, your isolated proteins are likely contaminated by cells.*

## Recipes

1. CH-medium
   50 ml solution G (see below)
   0.05 ml 0.1 M $CaCl_2$
   0.02 ml 1 M $MgSO_4 \cdot 7H_2O$
   0.1 ml 0.0475 M $MnSO_4$
   0.5 ml 2 mg/ml tryptophan
   Sterilize by filtering using Acrodisc® syringe filters; prepare freshly

 

2. Solution G

   25.0 g casein hydrolysate

   9.2 g L-glutamic acid

   3.125 g L-alanine

   3.48 g L-asparagine

   3.4 g $KH_2PO_4$

   1.34 g $NH_4Cl$

   0.27 g $Na_2SO_4$

   0.24 g $NH_4NO_3$

   2.45 mg $FeCl_3 \cdot 6H_2O$

   dd$H_2O$ 2.35 L and adjust pH to 7.0 by using 10 N NaOH

   Sterilize by autoclaving; stored at 4 °C

3. Resuspension Buffer

   5 mM $MgCl_2$

   50 mM Tris-HCl (pH 7.4)

## Acknowledgments

This protocol is adapted from Bach and Bramkamp (2013).

## References

1. Bach, J. N. and Bramkamp, M. (2013). Flotillins functionally organize the bacterial membrane. *Mol Microbiol* 88(6): 1205-1217.

 