# PROTOCOL FOR: Optimized transformation, overexpression and purification of S100A10

Authors

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# REAGENTS AND MATERIALS

pGEX-6P-1 (S100A10-GST) (Rezvanpour, Phillips et al. 2009)

*E. coli* BL21-CodonPlus (DE3)-RIL Competent Cells (Agilent Technologies, Canada, # 230245)

XL10-Gold β-mercaptoethanol (Agilent Technologies, Canada)

Tryptone (Fisher Scientific, NH, USA)

Yeast extract (Fisher Scientific, NH, USA)

NaCl (VWR International, PA, USA)

MgCl2 hexahydrate (Fisher Scientific, NH, USA)

MgSO4, anhydrous (VWR International, PA, USA)

D-Glucose, anhydrous (Bio Basic, ON, Canada)

Ampicillin sodium salt (Fisher Scientific, NH, USA)

Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Fisher Scientific, NH, USA)

Glycerol (Fisher Scientific, NH, USA)

Lysozyme (VWR International, #97062-136, PA, USA)

KCl (VWR International, PA, USA)

Na2HPO4, anhydrous (VWR International, PA, USA)

KH2PO4 (VWR International, PA, USA)

Tris base (Fisher Scientific, NH, USA)

Reduced glutathione (Fisher Scientific, NH, USA)

Ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate (Fisher Scientific, NH, USA)

Dithiothreitol (DTT) (Fisher Scientific, NH, USA)

30% Acrylamide/Bis solution (Bio-Rad Laboratories, CA, USA)

Sodium dodecyl sulfate (SDS) (Fisher Scientific, NH, USA)

Glycine (Fisher Scientific, NH, USA)

Bromophenol blue (Bio Basic, ON, Canada)

2-mercaptoethanol, 14.2 M (Bio-Rad Laboratories, CA, USA)

Ammonium persulfate (APS) (Fisher Scientific, NH, USA)

Coomassie brilliant blue R-250 (Bio Basic, ON, Canada)

Ethanol 100% (Greenfield Global, ON, Canada)

Acetic acid, glacial (VWR International, PA, USA)

PreScission protease (PSP) (Cedarlane Laboratories, #Z02799-250, ON, Canada)

SeeBlue Pre-Stained Protein Standard (Fisher Scientific, #LC5625, NH, USA)

High-Range Rainbow Molecular Weight Markers (VWR International, #CA95040-112LP, PA, USA)

# PROCEDURE

Transformation

1. Thaw 100 µL of *E. coli* BL21-CodonPlus (DE3)-RIL Competent Cells on ice and prepare 10 µL of 1:10 (v/v) XL10-Gold β-mercaptoethanol:ultrapure water.

2. Add 100 µL of the cells and 2 µL of diluted XL10-Gold β-mercaptoethanol into a 14 mL culture tube with a round bottom, mix gently with pipette and incubate on ice for 10 min while shaking gently every 2 min.

3. Add 50 ng of pGEX-6P-1 (S100A10-GST) into the tube and mix gently with a pipette. Incubate on ice for 30 min.

4. Give the sample a 20 sec heat pulse at 42 °C using a water bath. Then, incubate on ice for 2 min.

5. Immediately add 900 µL of SOC medium preheated at 42 °C.

6. Incubate the sample at 37 °C, 250 rpm for 1 h.

7. Spread 100 µL of the bacteria culture on a LB ampicillin agar plate. Incubate at 37 °C overnight (16 hrs).

***REST:*** When colonies appear on the agar plate, the plate can be kept at 4 °C for several days.

***ATTENTION:*** 20 sec heat pulse is very important to obtain a high colony number. Using a culture tube with a round bottom is also very important for the heatpulse step and another kind of bottom shape could influence the transformation result.

\* ***HINT:*** Mix the cells with pipette without making bubbles, because it could kill them.

Overexpression

8. Choose one colony and put into a 14 mL culture tube containing 4 mL of 2YT ampicillin medium.

9. Incubate this bacteria preculture at 37 °C, 250 rpm overnight (16 hrs).

***REST:*** After the overnight incubation, bacteria preculture can be kept at 4 °C for several hours until the bacteria culture steps.

10. Put 200 µL of bacteria preculture into 50 mL LB ampicillin medium preheated at 37 °C, incubate this bacteria culture at 37 °C, 250 rpm. The rest of bacteria preculture can be kept at 4 °C if it subsequently needs to be frozen.

11. Take 300 to 500 µL of bacteria culture every 20 to 30 mins to measure the optical density (O.D.) at 600 nm, until O.D.600 nm reaches 0.8. Start the induction of overexpression by immediately adding 0.5 mL 100 mM IPTG to have a final IPTG concentration of 1 mM.

12. Incubate the bacteria culture at 21 °C, 250 rpm overnight (16 hrs).

***ATTENTION:*** Add IPTG at an O.D.600 nm of 0.8 helps to get a better overexpression with more soluble S100A10-GST. Choosing another O.D.600 nm value could influence the overexpression result.

Bacteria cell lysis

13. Centrifuge the bacteria culture using the centrifuge #1 at 3,270 g, 4 °C for 30 mins. Only recover the cell pellet.

***REST:*** The cell pellet could be frozen in the centrifuge tube at -20 °C for several months.

14. Add 18.75 mL of PBS (1X) and 1.25 mL of lysozyme (4 mg/mL), then keep the tube on ice for 30 mins.

15. Make 3 freeze-thaw cycles.

16. Keep the tube on ice. Insert the probe of the sonicator into the sample and make 3 min of discontinuous sonication: 5 sec “on”, 5 sec “off”. At the end of the 3 min, make 30 sec of continuous sonication.

17. Use the centrifuge #2 to centrifuge sample at 15 000 g, 4 °C for 30 mins. Transfer the supernatant in another tube. Suspend the cell pellet into 20 mL PBS (1X).

***ATTENTION:*** the supernatant can only be kept at 4 °C for a maximum of 7 days. After 7 days, protein precipitation was noticed.

Check the overexpression results by SDS-PAGE (12%)

18. Mix 10 µL of sample and 2 µL of sample buffer, heat at 99 °C for 5 mins.

19. 10 µL of molecular marker (High-Range Rainbow Molecular Weight Markers) is loaded in one well. 10 µL of the different sample are also loaded in the other wells.

20. Run the SDS-PAGE at 200 V for 45-55 mins.

21. Rinse the gel 3 times with ultrapure water. The gel is then colored by the Coomassie blue solution for at least 2 h.

22. Then gel is rinsed 1 time with ultrapure water, and then discolored in the discoloration solution. The first discoloration takes at least 1 h and the second is left until the protein strip can be clearly observed on the gel.

Freeze the bacteria precultures that show successful overexpression results

23. Gently homogenize the preculture with a pipette without making bubbles.

24. Add glycerol to get a 1:1 (v/v) solution of glycerol:preculture. Mix gently.

25. The mixture is aliquoted in 2 mL, then put on dry ice until it is frozen.

26. The aliquots are kept at -80 °C.

\* ***HINT:*** If it is needed to start an overexpression with frozen bacteria preculture, only 50 µL is needed for 4 mL of 2YT ampicillin. Then, after incubation at 37 °C, 250 rpm overnight, the new preculture could be used for overexpression.

Purification of S100A10-GST

27. Use a GSTrap FF (1 mL) column as the first affinity chromatography at 4 °C.

28. The column is conditioned with at least 5 mL of PBS (1X) at a speed of 1 mL/min.

29. Pump the supernatant into the column at 0.2 to 0.5 mL/min to have the best yield of purified S100A10-GST.

30. Wash the column with PBS (1X) at 1 mL/min, collect the fractions and measure the O.D. at 280 nm and 260 nm. Stop washing the column when the absorbance is around zero or reaches a constant baseline.

31. Elute the column with the elution buffer at 0.5 mL/min, collect the fraction and measure the O.D. at 280 nm and 260 nm. Stop the elution when the absorbance is around zero.

Check the results of S100A10-GST purification by SDS-PAGE (12%)

32. Same steps as steps 18 to 22.

Elimination of reduced glutathione with buffer-exchange centrifugal filtration

33. Bring together all the fractions containing the purified S100A10-GST, measure the O.D. at 280 nm and 260 nm, and calculate the quantity of protein that was recovered.

34. Use the centrifuge #1 to concentrate the sample by centrifugation with a 3 kDa filter at 3270 g, 4 °C, 1 hr. The concentrated sample should be around 1 mL or less.

35. Add 14 mL of the basic filtration buffer, concentrate the sample to around 1 mL or less.

36. Repeat this step 3 more times.

37. Add 14 mL of the basic filtration buffer, incubate the sample at 4 °C overnight.

38. Repeat 4 more times steps 27 to 29.

39. Concentrate the sample to around 1 mL or less, then add 14 mL of the cleavage buffer.

40. Concentrate the sample to around 1 mL or less, then complete to 2 mL with the cleavage buffer.

41. Measure the O.D. at 280 nm and 260 nm to calculate the quantity of protein that was recovered.

\* ***HINT:*** It is important to calculate the protein amount before and after using buffer-exchange centrifugal filtration because the filter may retain some of the proteins in the sample.

Cleavage of the GST tag

42. Add PSP (1 I.U.: 0.12 mg S100A10-GST) to the concentrated sample in a tube.

43. Shake the sample gently using an orbital shaker at 4 °C for 2 h.

Purification of the S100A10

44. Use a GSTrap HP (1 mL) column as the first affinity chromatography at 20 °C.

45. The column is conditioned with at least 5 mL of PBS (1X) at a speed of 1 mL/min.

46. Pump the cleaved sample into the column at 0.2 to 0.5 mL/min to have the best yield of purified S100A10. Collect the fractions at the same time.

47. Wash the column with PBS (1X) at 1 mL/min, collect the fractions and measure the O.D. at 280 nm and 260 nm. Stop washing the column when the absorbance is around zero or reaches a constant baseline.

\* ***HINT:*** Using a small speed to pump the cleaved sample into the column helps to reach a high purity of S100A10.

Check the results of S100A10 purification by SDS-PAGE (12%)

48. Same steps as steps 18 to 22.

# RECIPES

LB medium (Sterilized): Tryptone 10 g/L, yeast extract 5 g/L and NaCl 10 g/L.

SOB medium (Sterilized): Tryptone 20 g/L, yeast extract 5 g/L, NaCl 0.5 g/L., MgCl2 0.01 M and MgSO4 0.01 M.

SOC medium (Sterilized): SOB medium with 0.02 M of glucose.

Ampicillin solution (Sterilized): 100 g/L.

LB ampicillin agar medium (Sterilized): Tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, agar 20 g/L and ampicillin 0.1 g/L.

LB ampicillin medium (Sterilized): Tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and ampicillin 0.1 g/L.

2YT medium (Sterilized): Tryptone 16 g/L, yeast extract 10 g/L, NaCl 5 g/L and ampicillin 0.1 mg/ml.

IPTG solution (Sterilized): 100 mM.

Lysozyme solution: 4 mg/mL, 1.25 mL/aliquot, store at -20 °C.

Glycerol solution: 90 % glycerol (v/v), MgSO4 0.1 M, Tris base 0.025 M.

PBS (10X): NaCl 1400 mM, KCl 27 mM, Na2HPO4 100 mM, KH2PO4 18 mM and pH 6.8.

PBS (1X): NaCl 140 mM, KCl 2.7 mM, Na2HPO4 10 mM, KH2PO4 1.8 mM and pH 7.3.

Elution buffer: Tris base 50 mM, reduced glutathione 20 mM and pH 8.0.

Cleavage buffer (for PSP): Tris base 50 mM, NaCl 150 mM, EDTA 1 mM, DTT 1 mM and pH 7.0.

Basic filtration buffer: Tris 50 mM, NaCl 100 mM and pH 9,5

SDS-PAGE running buffer (10X): SDS 10 g/L, Tris base 30.3 g/L, glycine 144 g/L.

SDS-PAGE running buffer (1X): 1/10 dilution of the 10X SDS-PAGE running buffer.

SDS solution: 10 % (w/v).

Tris-HCl solution 1.5 M, pH 8.8.

Tris-HCl solution 1.5 M, pH 6.8.

APS solution: 10 % (w/v), must be prepared freshly to use.

Sample buffer: Tris base 0.35 M at pH 6.8, SDS 10.28% (w/v), glycerol 34 % (v/v), 2-mercaptoethanol 5% (v/v) and bromophenol blue 0.012% (w/v).

Coomassie blue solution: Coomassie brilliant blue R-250 1 g/L, acetic acid 10 % (v/v), ethanol 40 % (v/v).

Discoloration solution: acetic acid 10 % (v/v), ethanol 40 % (v/v).

# EQUIPMENT

Water bath (Thermo NESLAB, NH, USA)

Incubator for agar plate (VWR International, PA, USA)

Incubator for bacteria culture (Forma Scientific, OH, USA)

14 mL culture tube with round bottom (Fisher Scientific, NH, USA)

Spectrophotometer (O.D. measurement) (Eppendorf, Hamburg, Germany)

Centrifuge #1 (Beckman Coulter, CA, USA)

Sonicator (Fisher Scientific, NH, USA)

Centrifuge #2 (Thermo Scientific, MA, USA)

Mini heat-bloc (VWR International, PA, USA)

Electrophoresis equipment (SDS-PAGE) (Bio-Rad Laboratories, CA, USA)

GSTrap FF (1 ml) (GE Healthcare, IL, USA)

GSTrap HP (1 ml) (GE Healthcare, IL, USA)

Pump (for chromatography) (VWR International, PA, USA)