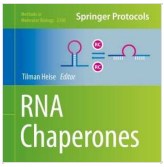

Sep 02, 2021

Purification of the Recombinant RNA Chaperone CspA

Book Chapter

In 1 collection

Pilar Menendez-Gil[1], Carlos J. Caballero[1], Cristina Solano[2], Alejandro Toledo-Arana[1]

[1]Instituto de Agrobiotecnología, IDAB, CSIC-UPNA-Gobierno de Navarra, Pamplona, Navarra, Spain;
[2]Navarrabiomed-Universidad Pública de Navarra (UPNA)-Complejo Hospitalario de Navarra (CHN), IDISNA, Pamplona, Navarra, Spain

1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.bnijmccn

Springer Nature Books

satyavati Kharde

ABSTRACT

This is part 3.1 of the "Fluorescent Molecular Beacons Mimicking RNA Secondary Structures to Study RNA Chaperone Activity" collection of protocols.

Collection Abstract: Molecular beacons (MBs) are oligonucleotide probes with a hairpin-like structure that are typically labelled at the 5' and 3' ends with a fluorophore and a quencher dye, respectively. The conformation of the MB acts as a switch for fluorescence emission. When the fluorophore is in close proximity to the quencher, fluorescence emission cannot be detected, meaning that the switch is in an OFF state. However, if the MB structure is modified, separating the fluorophore from the quencher, the switch turns ON allowing fluorescence emission. This property has been extensively used for a wide variety of applications including real-time PCR reactions, study of protein-DNA interactions, and identification of conformational changes in RNA structures. Here, we describe a protocol based on the MB technology to measure the RNA unfolding capacities of the CspA RNA chaperone from *Staphylococcus aureus*. This method, with slight variations, may also be applied for testing the activity of other RNA chaperones, RNA helicases, or ribonucleases.

DOI

dx.doi.org/10.17504/protocols.io.bnijmccn

EXTERNAL LINK

https://link.springer.com/protocol/10.1007/978-1-0716-0231-7_3

PROTOCOL CITATION

Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana 2021. Purification of the Recombinant RNA Chaperone CspA. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnijmccn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Menendez-Gil P., Caballero C.J., Solano C., Toledo-Arana A. (2020) Fluorescent Molecular Beacons Mimicking RNA Secondary Structures to Study RNA Chaperone Activity. In: Heise T. (eds) RNA Chaperones. Methods in Molecular Biology, vol 2106. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0231-7_3

COLLECTIONS

Fluorescent Molecular Beacons Mimicking RNA Secondary Structures to Study RNA Chaperone Activity

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

KEYWORDS

RNA, Chaperone, RNA-binding protein, Hairpin, Stem loop, Molecular beacon, Fluorescein, Quencher, FAM

CREATED

Oct 16, 2020

LAST MODIFIED

Sep 02, 2021

OWNERSHIP HISTORY

Oct 16, 2020	Lenny Teytelman	protocols.io
Jul 05, 2021	Emma Ganley	protocols.io
Aug 24, 2021	Satyavati Kharde	
Aug 26, 2021	satyavati Kharde	
Aug 26, 2021	satyavati Kharde	

PROTOCOL INTEGER ID

43307

PARENT PROTOCOLS

Part of collection

Fluorescent Molecular Beacons Mimicking RNA Secondary Structures to Study RNA Chaperone Activity

MATERIALS TEXT

Prepare all solutions using ultrapure water (prepared by purifying deionized water to reach a sensitivity of at least 18 MΩ at **25 °C**) and analytical grade reagents for use in molecular biology. Store solutions at **Room temperature** unless stated otherwise. Follow safety and waste disposal regulations when handling harmful products accordingly.

Materials for section "*Growth of Bacteria Expressing GST-CspA Fusion Protein*":

1. *E. coli* BL21 (DE3) harboring pGEX-6P-2::*cspA* (*see* **Note 1**).
2. Sterile material for bacterial growth: 10-, 100- and 1000-µL pipette tips, test tubes, 2-L Erlenmeyer flasks, graduated cylinders, 250-mL centrifuge tubes, petri dishes, 1.5-mL Eppendorf tubes.
3. [M]**100 mg/mL ampicillin stock solution** sterilized by filtration. Store at **-20 °C**.
4. [M]**40 % Glucose solution** sterilized by filtration.
5. Luria Bertani (LB) agar plates supplemented with [M]**100 µg/mL ampicillin**.
6. Microbiological incubator at **37 °C**.
7. Luria Bertani (LB) medium sterilized by autoclave and supplemented with glucose and ampicillin to a final concentration of [M]**1 %** and [M]**100 µg/mL**, respectively.

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

8. Shaking incubator at **37 °C** and **200 rpm** .
9. Spectrophotometer.
10. Centrifuge with rotor for Eppendorf tubes.
11. **200 mg/mL Isopropyl-β-D-1-thiogalactopyranoside (IPTG) stock solution** , sterilized by filtration. Store at **-20 °C** .
12. Refrigerated centrifuge with rotor for 250-mL centrifuge flasks.
13. Phosphate buffered saline (PBS): **7.3** , **140 Milimolar (mM) NaCl** , **2.7 Milimolar (mM) KCl** , **10 Milimolar (mM) Na_2HPO_4**, **1.8 Milimolar (mM) KH_2PO_4** sterilized by autoclave.

Materials for section "*Bacterial Cell Lysis and Recovery of Total Protein Crude Extract*":

1. Sterile 15-mL and 50-mL conical tubes.
2. Sterile PBS **7.3** (*see* **item 13** in Materials above).
3. **50 mg/mL Lysozyme stock solution** sterilized by filtration. Store at **-20 °C** .
4. **10 mg/mL RNase A stock solution** . Store at **-20 °C** .
5. **100 Milimolar (mM) Phenylmethanesulfonyl fluoride (PMSF) stock solution** prepared in isopropanol. Store at **-20 °C** (*see* **Note 2**).
6. Shaking incubator at **30 °C** and **200 rpm** .
7. Branson sonifier 250 with microtip.
8. Centrifuge with a rotor for 50-mL tubes, which allows centrifugation at **16000 x g**
9. High speed centrifuge tubes.
10. **5 mg/mL DNase I stock solution** prepared by dissolving DNase I powder in **0.15 Molarity (M) NaCl** .
11. 0.45 µm filters and 1.5-mL Eppendorf tubes.
12. **12 % SDS-polyacrylamide gels** .
13. **6 X Sample buffer** : **375 Milimolar (mM) Tris–HCl** **6.8** , **9 % SDS** , **50 % glycerol** , **9 % β-mercaptoethanol** and **0.03 % bromophenol blue** . Store at **-20 °C** .
14. Tris–glycine running buffer: **25 Milimolar (mM) Tris** , **192 Milimolar (mM) glycine** , **0.1 % SDS** .
15. Protein molecular weight marker. Store at **-20 °C** .
16. Heating block.
17. Electrophoresis chamber for polyacrylamide gels.
18. Power supply.
19. Coomassie brilliant blue R250 solution.
20. Orbital shaker.
21. Destaining solution: **40 % ethanol** and **10 % acetic acid** in water.
22. Fixation solution: **10 % ethanol** and **3 % glycerol** in water.

Materials for section "*Purification of Recombinant CspA from Total Protein Crude Extracts*":

1. AKTAprime plus chromatography system.
2. GSTrap FF 5-mL column.
3. GSTrap FF 1-mL column.
4. HiPrep 16/60 Sephacryl S-100 HR column.
5. Ultrapure water, sterilized by autoclave and degassed.
6. **20 % ethanol solution** sterilized by autoclave and degassed.
7. Binding Buffer: degassed sterile PBS **7.3** (*see* **item 13** in Materials for section "*Growth of Bacteria Expressing GST-CspA Fusion Protein*").

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

8. PreScission Protease buffer: [M]**50 Milimolar (mM) Tris–HCl** pH**7**, [M]**150 Milimolar (mM) NaCl**, [M]**1 Milimolar (mM) EDTA**, [M]**1 Milimolar (mM) DTT** sterilized by autoclave and degassed.
9. PreScission Protease.
10. PreScission Protease mix: mix **100 µl (200 units) PreScission Protease** with **4.9 mL PreScission Protease buffer** at **4 °C**.
11. 5-mL syringe with Luer tip.
12. 1.5-mL Eppendorf tubes and 15-mL conical tubes.
13. Elution buffer: [M]**50 Milimolar (mM) Tris–HCl** pH**8**, [M]**10 Milimolar (mM) reduced glutathione** sterilized by autoclave and degassed.
14. Gel Filtration buffer: [M]**20 Milimolar (mM) Tris–HCl** pH**7.4**, [M]**500 Milimolar (mM) NaCl**.
15. Slide-A-Lyzer Dialysis Cassettes.
16. 0.22 µm filters.
17. CspA Storage buffer: [M]**10 Milimolar (mM) Tris–HCl** pH**8**, [M]**1 Milimolar (mM) EDTA**, [M]**50 Milimolar (mM) potassium chloride** and [M]**10 % glycerol**.
18. Bio-Rad protein assay.
19. 96-well standard plates.
20. MultiSkan EX (Labsystems) or any other equivalent microplate photometer.

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

Prepare Buffers and Solutions as described in section 'Materials'.

Growth of Bacteria Expressing the GST-CspA Fusion Protein 5h

1

Streak the *E. coli* BL21 (DE3) pGEX-6P-2::*cspA* strain in an LB agar plate supplemented with [M]**100 µg/mL ampicillin**.

2

Incubate at **37 °C** **Overnight**.

3 Inoculate a colony of the previous culture into a sterile test tube containing LB medium supplemented with [M]**100 µg/mL ampicillin** and [M]**1 % glucose**.

4

Grow culture at **37 °C** and **200 rpm** **Overnight**.

5 Inoculate **500 µl of the bacterial preculture (1/1000 dilution factor)** into two sterile pre-warmed 2-L Erlenmeyer flasks containing **500 mL LB medium** supplemented with [M]**100 µg/mL ampicillin** and [M]**1 % glucose**.

6

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

Mix and incubate the cultures at **37 °C** and **200 rpm** until an optical density (OD_{600nm}) of 0.5 is reached.

7 Induce the expression of CspA by addition of IPTG to a final concentration of **0.4 Milimolar (mM)** .

8

Save **1 mL of culture of one of the flasks** and centrifuge it at **18000 x g, 00:03:00** . Store the bacterial pellet at **-20 °C** . This aliquot sample corresponds to the pre-induction control (*see* **Note 9**).

9 5h

Resume bacterial growth for another **05:00:00** at **37 °C** and **200 rpm** .

10

Save **1 mL of culture of one of the flasks** and centrifuge it at **18000 x g, 00:03:00** . Store the bacterial pellet at **-20 °C** (post-induction control) (*see* **Note 9**).

11

Harvest the rest of the cultures in 250-mL tubes and centrifuge at **5000 x g, 00:10:00** (*see* **Note 10**).

12 Discard the supernatant and resuspend the pellets in **1 volume PBS, pH 7.3** .

13

Repeat the centrifugation step, discard the supernatant and store the bacterial pellets at **-80 °C** (*see* **Note 11**).

Bacterial Cell Lysis and Recovery of Total Protein Crude Extract 1h 1m

14

Thaw the bacterial pellets, resuspend them in **25 mL PBS, pH 7.3** in 50-mL conical tubes (per pellet) and add lysozyme, RNase and PSMF to a final concentration of **1 mg/mL** , **10 µg/mL** , and **1 Milimolar (mM)** , respectively.

15 30m

Incubate the samples for **00:30:00** at **30 °C** and **200 rpm** .

16 Sonicate the samples **On ice** as follows: 3 cycles of **00:00:30** power 4, 2 cycles of **00:00:30** power 5. 1m

Leave the samples **On ice** for **00:01:00** in between cycles

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

Leave the samples On ice for 00:01:00 in between cycles.

17 

Centrifuge the samples at 16000 x g, 4°C, 00:30:00 (*see* **Note 10**).

18 Transfer the supernatant (soluble fraction) to new tubes and store the pellet at -20 °C .

Pellets (insoluble fraction) contain inclusion bodies (IB), and constitute the IB control (*see* **Note 9**).

19 Supplement the soluble fraction with DNase I and RNase A to a final concentration of [M]10 µg/mL and [M]5 µg/mL , respectively.

20 

30m

Incubate On ice for 00:30:00 .

21 Store 50 µl of the sample at -20 °C (pre-filtered soluble fraction control) (*see* **Note 9**).

22 Filter the soluble fraction using a 0.45 µm filter whilst On ice (*see* **Note 12**).

23 Store 50 µl of the sample at -20 °C (post-filtered soluble fraction control) and the rest of the soluble fraction at -20 °C (*see* **Note 9**).

24 

Mix aliquots of the different control samples (pre-induction control, post-induction control, IB control, pre-filtered soluble fraction and post-filtered soluble fraction), collected in the previous steps (*see* **Note 9**), with [M]6 X sample buffer to a final concentration of [M]1 X .

25 Denature mixtures at 95 °C for 00:05:00 and load them in a polyacrylamide gel (a Molecular Weight Marker should be included) (*see* **Note 13**).

5m

26 Run the gel with [M]1 X running buffer at 130 V until the front reaches the bottom of the gel (*see* **Note 14**).

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

27 Stain the gel with Coomassie blue for at least **04:00:00** at **Room temperature** on an orbital shaker. 4h

28 15m

Destain the gel with several washes of destaining solution at **Room temperature** and shaking. Once protein bands are visible and the background level is low, incubate the gel with fixing solution for **00:15:00** at **Room temperature** and shaking.

Optimal results are reached when most of the GST-CspA fusion protein appears in the soluble fraction and not in the inclusion bodies fraction (*see* **Note 15**).

Purification of the Recombinant CspA Protein 30m

29 Thaw the post-filtered soluble fraction and purify the GST-CspA fusion protein with the use of a GSTrap FF 5-mL column and an AKTAprime plus chromatography system, following the recommendations of the manufacturer.

30 Clean the system with **[M]20 % ethanol** and ultrapure water.

31 Connect the column to the AKTAprime plus system “drop to drop” to avoid introducing air into the column.

32 Equilibrate the column with **25 mL binding buffer** at a flow rate of **5 mL/min**.

33 Apply the sample at a flow rate of **0.2 mL/min** (*see* **Note 16**).

34

Wash the column with **50 mL binding buffer** at a flow rate of **5 mL/min**.

35 Equilibrate the column with **50 mL PreScission Protease buffer** at a flow rate of **5 mL/min** and disconnect the column from the AKTAprime plus chromatography system.

36 Prepare the PreScission Protease mix at **4 °C** and load it manually onto the column using a syringe at a flow rate of **1 mL/min**.

37 15m

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

Seal the column with the top and bottom stop plugs and incubate **Overnight** at **4 °C** .

38 Connect a GSTrap FF 1-mL column to the AKTAprime plus system and equilibrate it with **5 mL PreScission Protease buffer** at a flow rate of **1 mL/min**.

39 Place the GSTrap FF 5-mL column on top of the GSTrap FF 1-mL column.

This tandem column scheme acts as a filter to capture any released cleaved GST proteins, uncleaved GST-tagged proteins and unbound PreScission Protease.

40 Elute CspA with **15 mL PreScission Protease buffer** at a flow rate of **1 mL/min**. Collect 1 mL fractions containing the CspA protein and place them **On ice** .

41 Elute the GST and GST-PreScission Protease from the columns with **30 mL elution buffer** at a flow rate of **1 mL/min**.

42 Clean the system and columns with ultrapure water and **20 % ethanol** and remove columns from the system.

43 Dialyze the CspA fractions against Gel Filtration buffer using a Slide-A-Lyzer Dialysis Cassette **Overnight** at 15m **4 °C** .

44 Collect CspA from the Dialysis Cassette and filter the solution using a 0.22 µm filter. Keep the CspA sample **On ice** until its purification by size exclusion chromatography.

45 Connect a HiPrep 16/60 Sephacryl S-100 HR Column (*see* **Note 17**) to the AKTAprime plus system “drop to drop” to avoid introducing air into the column.

46 Equilibrate the column with **60 mL ultrapure water** at a flow rate of **0.5 mL/min** and then with **240 mL Gel Filtration buffer** at a flow rate of **1 mL/min**.

47 Inject the CspA sample into the column and run it with **120 mL Gel Filtration buffer** at a flow rate of **0.5 mL/min**. Collect 3 mL fractions and place them **On ice** .

48 Clean the column with **480 mL ultrapure water** and **480 mL 20% ethanol** at a flow rate of **1 mL/min**.

49 Remove the column from the system and clean the system with ultrapure water and **20 % ethanol** .

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn

50 To select fractions containing CspA, mix an aliquot of each peak fraction with sample buffer [M]**6 X** and perform a **12% PAGE** as described above.

51 Load the CspA selected fractions into a Slide-A-Lyzer Dialysis Cassette and dialyze against CspA Storage buffer 15m
Overnight at **4 °C** .

52 To assess protein purity, mix an aliquot of the recombinant CspA chaperone with sample buffer [M]**6 X** and perform a **12% SDS-polyacrylamide gel electrophoresis (PAGE)** as described above.

53 Determine the recombinant protein concentration by the Bio-Rad protein assay.

Citation: Pilar Menendez-Gil, Carlos J. Caballero, Cristina Solano, Alejandro Toledo-Arana (09/02/2021). Purification of the Recombinant RNA Chaperone CspA. https://dx.doi.org/10.17504/protocols.io.bnijmccn