



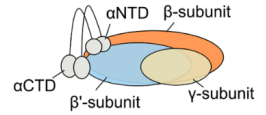
Jun 04, 2019

Version 2

# Purification of DNA-dependent RNA Polymerase from *Synechococcus elongatus* PCC 7942 V.2

DOI

[dx.doi.org/10.17504/protocols.io.3qcgmsw](https://dx.doi.org/10.17504/protocols.io.3qcgmsw)



Lutz LCB Berwanger<sup>1</sup>

<sup>1</sup>Heinrich-Heine Universität Düsseldorf



Lutz LCB Berwanger

Heinrich-Heine Universität Düsseldorf

## Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.3qcgmsw>

**Protocol Citation:** Lutz LCB Berwanger 2019. Purification of DNA-dependent RNA Polymerase from *Synechococcus elongatus* PCC 7942. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3qcgmsw>

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



**Protocol status:** Working

**We use this protocol and it's working**

**Created:** June 04, 2019

**Last Modified:** June 04, 2019

**Protocol Integer ID:** 24036

**Keywords:** RNA Polymerase, Cyano, 7942, Synechococcus, dependent rna polymerase from synechococcus elongatus pcc, synechococcus elongatus pcc, dependent rna polymerase, rna, pcc, dna

## Abstract

This protocol can be used to purify DNA-dependent RNA polymerase from *Synechococcus elongatus* PCC 7942 by heparin and ion-exchange chromatography.

## Materials

### MATERIALS

⊗ HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

⊗ Mono Q® 5/50 GL **GE Healthcare Catalog #GE17-5166-01**

### STEP MATERIALS

⊗ Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**

⊗ Benzonase® Nuclease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E1014 SIGMA**

⊗ HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

## Protocol materials

⊗ Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**

⊗ Benzonase® Nuclease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E1014 SIGMA**

⊗ HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

⊗ HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

⊗ Mono Q® 5/50 GL **GE Healthcare Catalog #GE17-5166-01**

⊗ Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**

⊗ Benzonase® Nuclease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E1014 SIGMA**

⊗ HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

## Troubleshooting

## Prepartions

### 1 Culture:

To get a reasonable amount of RNA polymerase it is recommended to use at least 8 litre ( $OD_{750nm} = 1$ ) culture or in other words 10 gramm of cell dry cell pellet.

### 2 Lysis buffer:

10mM Tris-HCl pH 7.9  
150mM NaCl  
1 protease inhibitor cocktail tablet (Roche) per 50ml  
0.1 mM DTT  
0.1 mM EDTA  
0.5% Glycerol

### 3 Loading buffer:

10 mM Tris-HCl pH 7.9  
150mM NaCl  
0.1 mM DTT  
0.1 mM EDTA  
0.5% Glycerol

### 4 Washing buffer:

10mM Tris-HCl pH 7.9  
300mM NaCl  
0.1 mM DTT  
0.1 mM EDTA  
0.5% Glycerol

### 5 Elution buffer:

10mM TrisHCl pH 7.9  
600mM NaCl  
0.1 mM DTT  
0.1 mM EDTA  
0.5% Glycerol

### 6 Column cleaning buffer:

10mM Tris-HCl pH 7.9  
2M NaCl

### 7 RNA polymerase storage buffer:

50% glycerol  
200mM KCl  
40mM Tris 7.9  
1mM EDTA  
1mM DTT

#### Note

All buffers are filtrated by vacuum pump filtration to avoid that air is getting into your HPLC system. Air bubbles will disturb your chromatogram detection.

## Sample Preparation

- 8 The dry pellets are resuspended in 25 mL lysis buffer. Add 0,5 µl of Benzonase® nuclease and 1 mg/mL lysozyme. Let the suspension incubate for 30 min on ice.

1d 6h

 Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #12671-19-1**

 Benzonase® Nuclease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E1014 SIGMA**

- 9 Sonicate the samples for 6 minutes of 2 second on/off at a 40 % amplitude.


6h

- 10 Cell Lysat undergoes two centrifugation steps of 15 minutes at 15000 rcf and 20 minutes at 18000 rcf until a clear supernatant is obtained. The supernatant was passed through a 0.45 µM PFDF filter.

15h

## Heparin Chromatography

- 11 Next step includes, using a high pressure liquid chromatography (HPLC). Heparin column was equilibrated with 5 column volumes (CV) of loading buffer. The lysate is loaded, and column is washed with ten CV of loading buffer. Followed by 10 CV of washing buffer. Elution is done by 10 CV of elution buffer. The column is washed with column cleaning buffer.

 HiTrap Heparin HP affinity column **GE Healthcare Catalog #17040701**

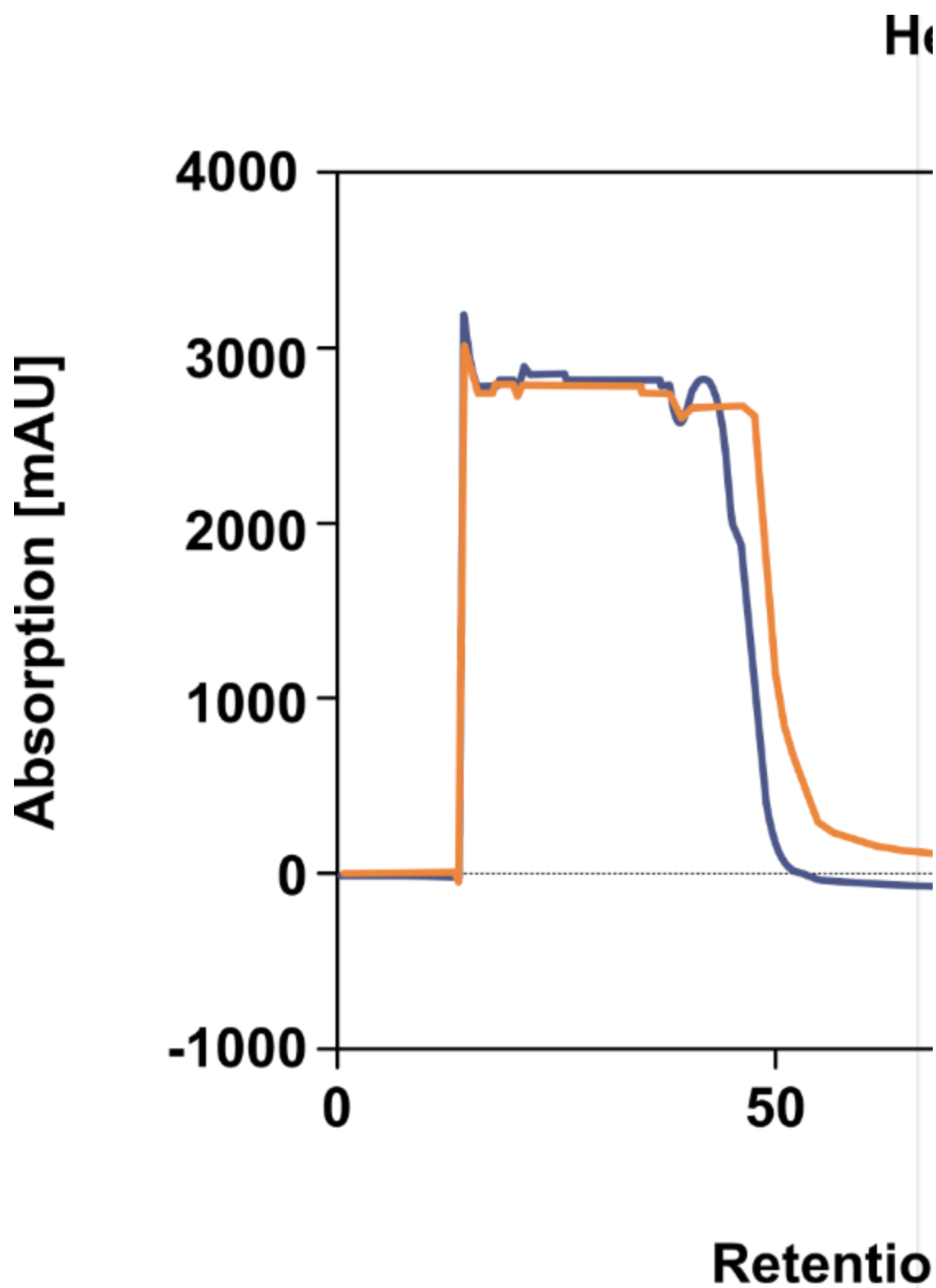
- 12 During elution step, one should see that the chromatogram peaks several times. These fractions are loaded onto SDS gel and visualized with comassie blue staining.



#### Note

An exemplary image of the heparin chromatogram is shown in the 'expected results' section. The star marked line indicates the peak of the RNA polymerase. Fractions around this peak are loaded onto a SDS gel.

Expected result



## Ion-exchange Chromatography

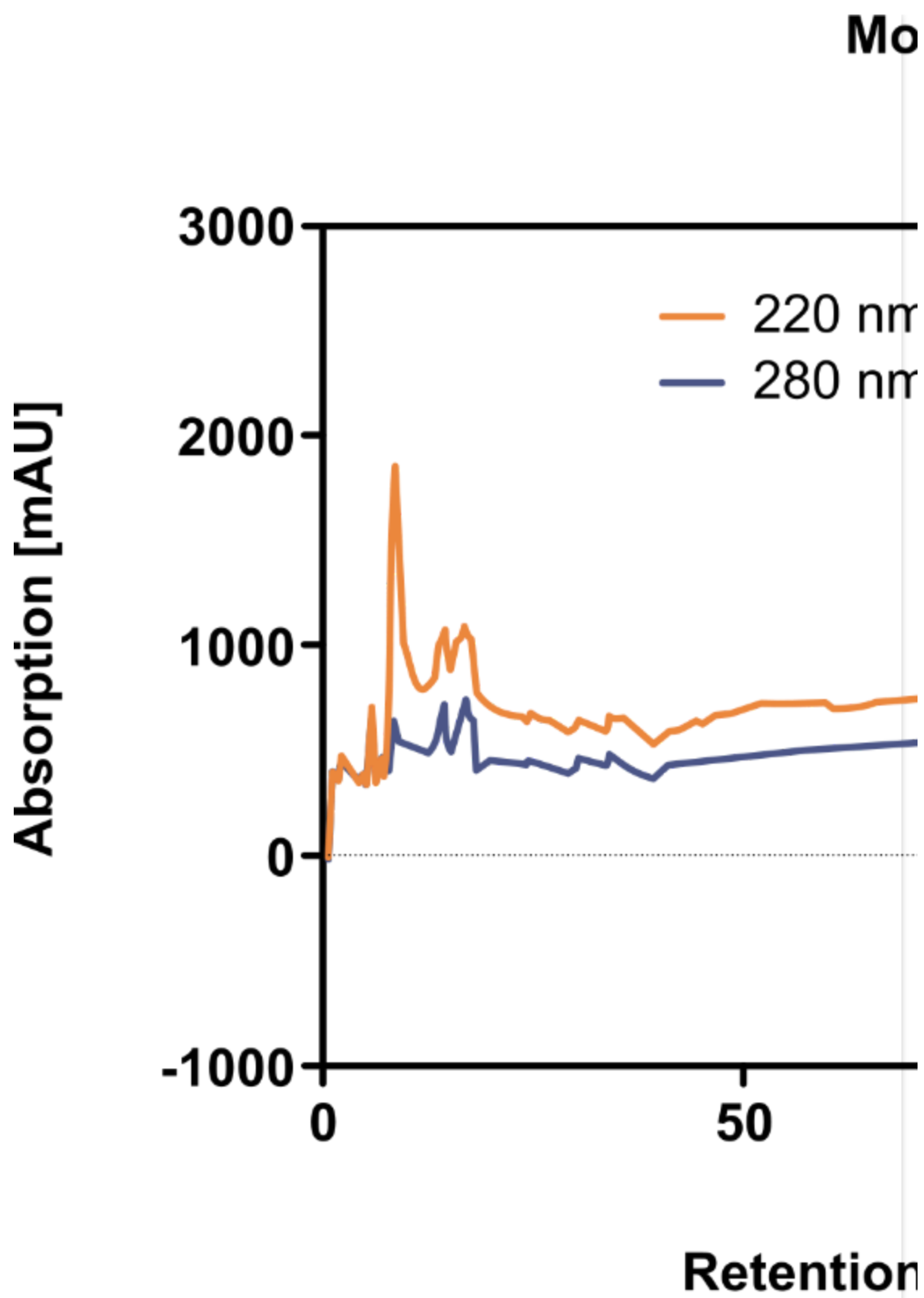
- 13 Fractions containing RNA polymerase were concentrated with Vivaspin® 500  $\mu$ L 5K ultrafiltration spin columns washed with loading buffer several times and subjected to further purification on an ion-exchange MonoQ 5/50 GL column, using fast protein liquid chromatography.

### Note

Washing step is crucial for further purification! One have to be sure that the elution buffer is fully exchanged with washing buffer to ensure binding of the RNA polymerase onto the ion-exchange column.

- 14 The column was equilibrated with five column volumes (CV) of loading buffer. Suspension was loaded onto column with loading buffer and continuously washed until the OD280nm reading drops to the baseline. Then a gradient of 50 CV from 250mM to 600mM NaCl [10mM TrisHCl pH 7.9, 0.1 mM DTT, 0.1 mM EDTA, 0.5% Glycerol] was applied. Final wash of the column is done with column cleaning buffer.

Expected result





#### Note

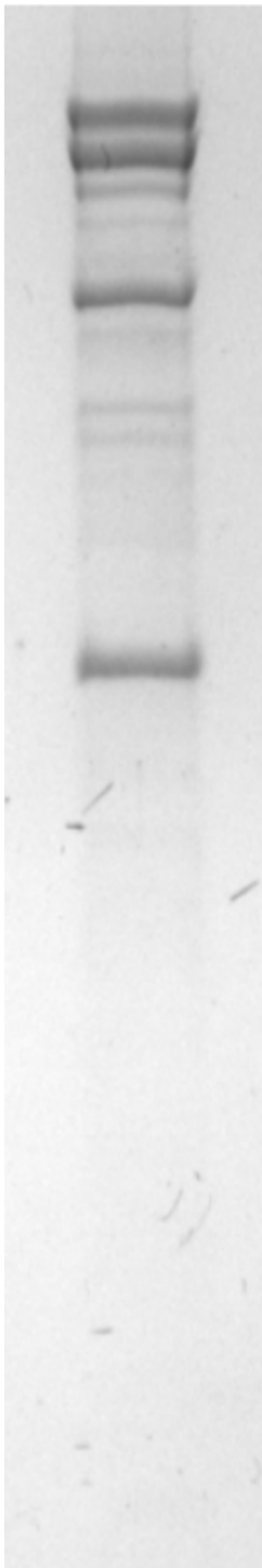
An exemplary image of the MonoQ chromatogram is shown in the 'expected results' section. The star marked line indicates the peak of the RNA polymerase. Fractions around this peak are loaded onto a SDS gel.

## RNA polymerase sample preparation

- 15 Again fractions containing RNA polymerase were concentrated with Vivaspin® 500  $\mu$ L 5K ultrafiltration spin columns washed with loading buffer several times. Use the same ultrafiltration spin column to change buffer and concentrate RNA polymerase with storage buffer. To validate your sample one can use western blotting or mass spectrometry. Samples are stored at  $-20^{\circ}\text{C}$ .



## Expected result





Typical result after Ion-exchange chromatography