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Working

Purification of DNA-dependent RNA Polymerase from *Synechococcus elongatus* PCC 7942

Version 2

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ABSTRACT

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

MATERIALS

NAME	CATALOG #	VENDOR
HiTrap Heparin HP affinity column	17040701	Ge Life Sciences
Mono Q® 5/50 GL	GE17-5166-01	Ge Life Sciences

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Lysozyme	12671-19-1	Sigma Aldrich
Benzonase® Nuclease	E1014 SIGMA	Sigma-aldrich
HiTrap Heparin HP affinity column	17040701	Ge Life Sciences

Preparations

- 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.
- 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
- 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



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

30h

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.



Lysozyme
by Sigma Aldrich
Catalog #: 12671-19-1



Benzonase® Nuclease
by 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

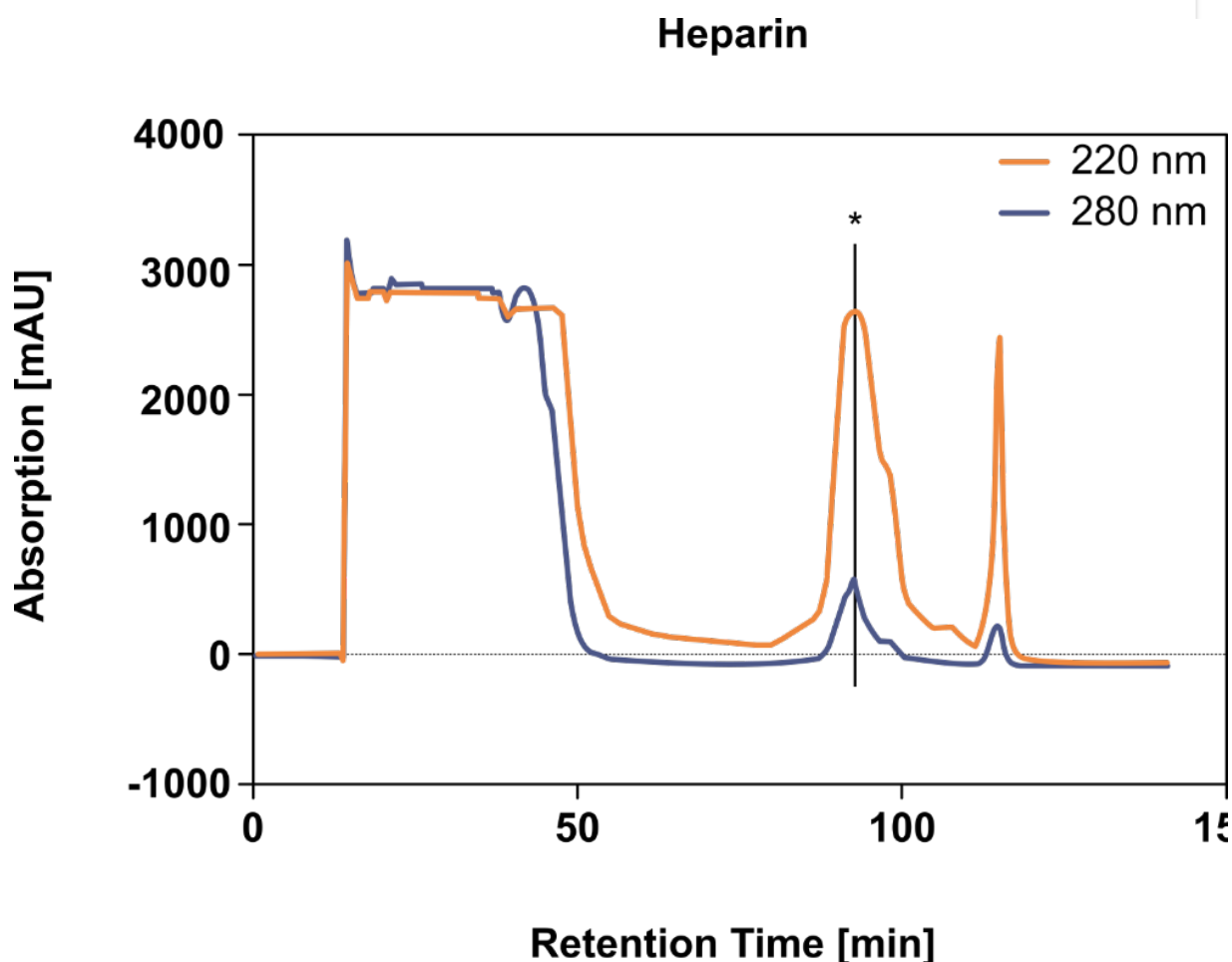
by GE Life Sciences

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.



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.



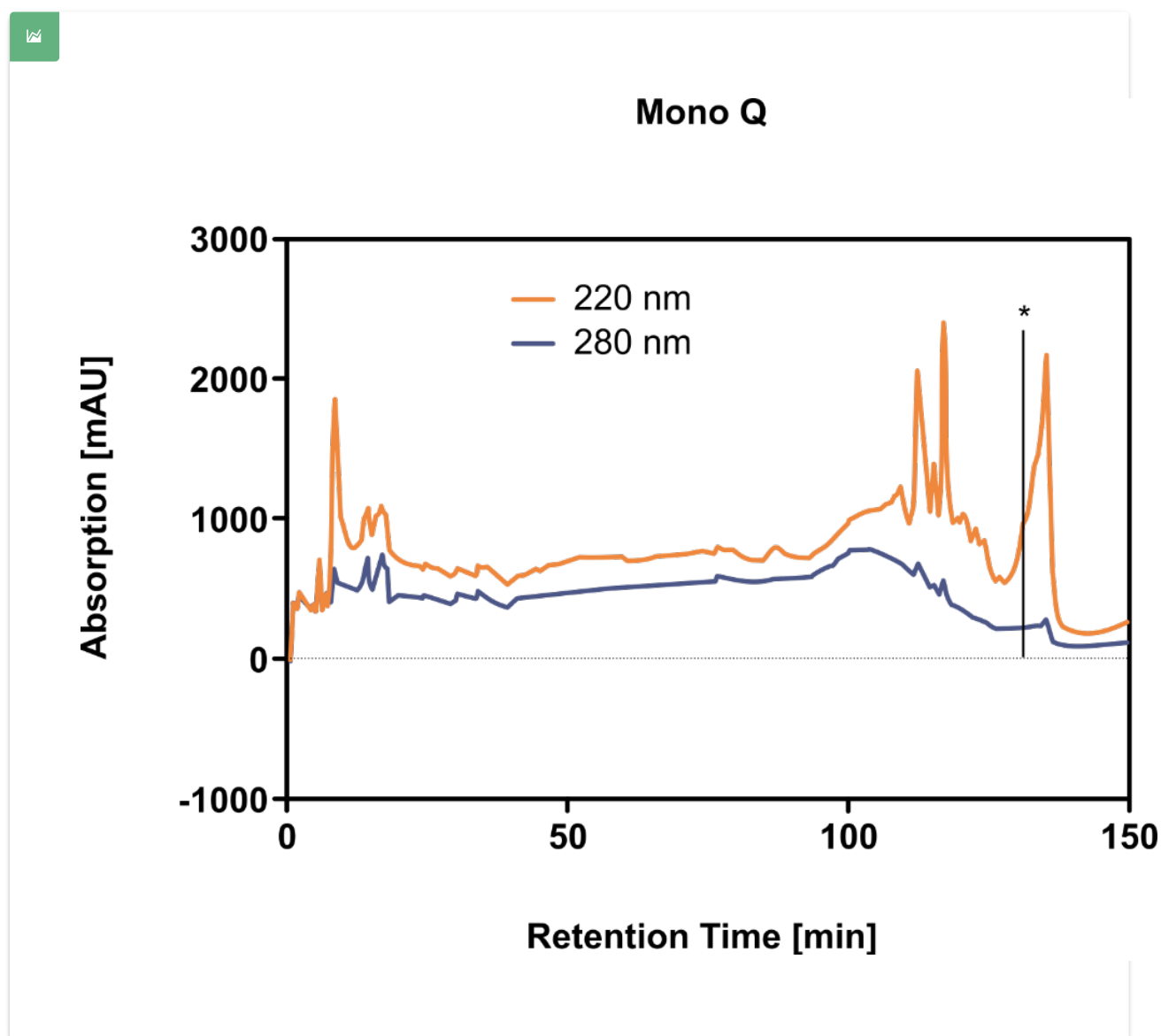
Ion-exchange Chromatography

- 13 Fractions containing RNA polymerase were concentrated with Vivaspin® 500 µL 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.



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 OD_{280nm} 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.



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 µL 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°C.



Typical result after Ion-exchange chromatography



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