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Purification of (Kai) proteins via size exclusion chromatography

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ABSTRACT

This protocol can be used to further purifiy (Kai) proteins via size exclusion chromatography using either HiPrep 16/60 Sephacryl S-300 HR column (120 ml column volume), HiPrep 16/60 Sephacryl S-200 HR (120 ml column volume) or Superdex 200 Increase 10/300 GL (24 ml column volume)

GUIDELINES

Choose the chromatography column depending on the amount and size of your protein. Purification can be performed at 4 °C or 30 °C depending on the stability of your protein.

MATERIALS

NAME	CATALOG #	VENDOR
MilliQ water		
Magnesium chloride hexahydrate	View	Sigma Aldrich
Tris(hydroxymethyl)aminomethane	252859-500G	Sigma Aldrich
NaCl		
HiPrep Sephacryl S-200 HR Column	17116601	Ge Healthcare
HiPrep Sephacryl S-300 HR Column	17116701	Ge Healthcare
Superdex 200 Increase 10/300 GL Column	28990944	Ge Healthcare
EtOH		
HCl	View	
14 Dithiotreitol (DTT)	6908.1	Carl Roth
Adenosin-5-triphosphate disodium salt (ATP)	HN35.1	Carl Roth
Quick Start™ Bradford 1x Dye Reagent	5000205	Bio-rad Laboratories

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Gel Filtration Standard	#1511901	BIO-RAD

MATERIALS TEXT

You will further need:

- reaction tubes
- 96 well plate
- centrifugal concentrators with appropriate MWCO
- chromatography system

preparation of buffer and solutions

1 Prepare 1 l of each:
- Degassed MiliQ
- Degassed 20 % EtOH
- Degassed running buffer [20-50 mM Tris/HCl (pH8), 150 mM NaCl, 1-2 mM DTT, *only for KaiC proteins: 5 mM $MgCl_2$, 1 mM ATP]*

set-up of your liquid chromatography system

2 Connect degassed MilliQ with pump A and pump B of your chromatography instrument

3 Purge and rinse all valves and sample loop with MilliQ (pump A and pump B)

4 Connect an appropriate sample loop (e.g. 2 ml) to your system and rinse with MilliQ

5 Connect a size exclusion chromatography column to your system (e.g. Superdex 200 Increase 10/300 GL, HiPrep 16/60 Sephacryl S-200 HR, HiPrep 16/60 Sephacryl S-300 HR column)

6 Wash column with at least 0.5 column volumes degassed MilliQ

Note: make sure not to exceed the maximal pressure the column can withstand. Recommended maximal flow rates:
- *for sephacryl S-200 and sephacryl S-300: 0.5 ml/min*
- *for superdex 200: 0.75 ml/min*

7 Connect running buffer as eluant A and purge

equilibration of the column

8 Equilibrate the column with at least 1.5 column volumes buffer A

Note: make sure not to exceed the maximal pressure the column can withstand. Recommended maximal flow rates:
- *for sephacryl S-200 and sephacryl S-300: 1 ml/min*
- *for superdex 200: 0.75 ml/min*

protein separation

9
- Remove aggregates and precipitates in your protein sample by centrifugation or filtration (use a syringe filter)
- Apply your protein to the sample loop (*injection valve must be set to load position*).

10 Separate in 1 column volume running buffer using the following flow rates:
- for sephacryl S-200 and sephacryl S-300: 0.4-0.5 ml/min
- for superdex 200: 0.75 ml/min

11 Shortly before void proteins will be eluted: start to collect fractions of 0.5 ml – 1 ml

12 *Note: to estimate the size of (the oligomeric states of) your proteins, separate a standard solution (e.g. Biorad gel filtration standard) under the same conditions*

Gel Filtration Standard
by BIO-RAD
Catalog #: #1511901

cleaning/storage

13 Wash with at least 1.5 column volumes MilliQ (pump B)

Note: make sure not to exceed the maximal pressure the column can withstand. Recommended maximal flow rates:
- *for sephacryl S-200 and sephacryl S-300: 0.4-0.5 ml/min*
- *for superdex 200: 0.75 ml/min*

Alternatively: wash with 1 column volume buffer, rinse with 0.5 column volumes MilliQ and equilibrate with 2 column volumes buffer for the next separation (in this case you can skip cleaning with EtOH described in steps 14 and 15)

14 Connect pump A to 20 % EtOH and purge

15 Wash with at least 1.5 column volumes 20 % EtOH (pump A)

Note: make sure not to exceed the maximal pressure the column can withstand. Recommended maximal flow rates:
for sephacryl S-200 and sephacryl S-300: 0.2 ml/min
for superdex 200: 0.4 ml/min

qualitative analysis of eluted fractions

16 Choose fractions of interest based on the absorption at 280 nm

17 For each fraction of interest, pipette 80 µl of Bradford solution in a well of a 96 well plate and add 5-20 µl of your fraction. Colour change to blue indicates that you successfully eluted proteins. Keep those fractions

18 Control homogeneity and size of your eluted protein(s) by separation via SDS-PAGE

19 Measure protein concentration in the fraction of interest (using e.g. Bradford method or infrared spectrometer (direct detect instrument, Merck))

Note: If necessary, you can concentrate your protein using a disposable centrifugal concentrator