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Purification of (Kai) proteins via size exclusion chromatography

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ABSTRACT

This protocol can be used to further purify (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.

Protocol status: Working
We use this protocol and it's working

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9363

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MATERIALS

MATERIALS

- ☒ MilliQ water **Contributed by users**
- ☒ Magnesium chloride hexahydrate **Sigma Aldrich**
- ☒ Tris(hydroxymethyl)aminomethane **Sigma Aldrich Catalog #252859-500G**
- ☒ NaCl **Contributed by users**
- ☒ HiPrep Sephacryl S-200 HR Column **Ge Healthcare Catalog #17116601**
- ☒ HiPrep Sephacryl S-300 HR Column **Ge Healthcare Catalog #17116701**
- ☒ Superdex 200 Increase 10/300 GL Column **Ge Healthcare Catalog #2899094**
- ☒ EtOH **Contributed by users**
- ☒ HCl **Contributed by users**
- ☒ 14 Dithiotreitol (DTT) **Carl Roth Catalog #6908.1**
- ☒ Adenosin-5-triphosphate disodium salt (ATP) **Carl Roth Catalog #HN35.1**
- ☒ Quick Start™ Bradford 1x Dye Reagent **Bio-rad Laboratories Catalog #5000205**

STEP MATERIALS

- ☒ Gel Filtration Standard **Bio-Rad Laboratories Catalog ##1511901**

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 MgCl₂, 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 **Bio-Rad Laboratories 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 μ l of Bradford solution in a well of a 96 well plate and add 5-20 μ l 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