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Purification of (Kai) proteins via size exclusion chromatography

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Protocol status: Working

We use this protocol and it's working

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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.

Materials

MATERIALS

 MilliQ water

 Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)**

 Tris(hydroxymethyl)aminomethane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #252859-500G**

 NaCl

 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 #28990944**

 EtOH

 HCl

 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



Protocol materials

⊗ Quick Start™ Bradford 1x Dye Reagent **Bio-Rad Laboratories Catalog #5000205**

⊗ HiPrep Sephacryl S-200 HR Column **GE Healthcare Catalog #17116601**

⊗ Superdex 200 Increase 10/300 GL Column **GE Healthcare Catalog #28990944**

⊗ MilliQ water

⊗ Adenosin-5-triphosphate disodium salt (ATP) **Carl Roth Catalog #HN35.1**

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

⊗ Tris(hydroxymethyl)aminomethane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #252859-500G**

⊗ NaCl

⊗ HiPrep Sephacryl S-300 HR Column **GE Healthcare Catalog #17116701**

⊗ EtOH

⊗ HCl

⊗ Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ 14 Dithiotreitol (DTT) **Carl Roth Catalog #6908.1**

preparation of buffer and solutions

- 1 Prepare 1 l of each:
 - Degassed MilliQ
 - 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