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Clusterin purification from HEK293E cells

Dr Patricia Yuste-Checa[1]

[1]Max Planck Institute of Biochemistry

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Felix Kraus

ABSTRACT

This protocol details the preocedure of clusterin purification from HEK293E cells.

ATTACHMENTS

dtisbiezx.pdf

DOI

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PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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MATERIALS TEXT

Buffers:

Binding buffer: [M]**20 Milimolar (mM)** Na acetate pH**5.0** .

Denaturing buffer:

A	B
Na acetate pH 5.0	20 mM
Urea	6 M

Elution buffer:

A	B
Na acetate pH 5.0	20 mM
NaCl	500 mM

Size exclusion chromatography buffer:

A	B
Na acetate pH 5.0	20 mM
NaCl	100 mM
EDTA	1 mM

FreeStyle™ 293 Expression Medium **Thermo Fisher Catalog #12338018**

Clusterin expression 4d

1 Express Clusterin (Clu) in HEK293E cells cultured in FreeStyle 293 Expression Medium (Thermo Fisher Scientific, 12338018) for **96:00:00** . 4d

Note: This protocol was optimized using HEK293E cells stably expressing Clu-Strep tag (pB-TPAF-CluStrep), however Clu without any affinity tag can be purified following this method since the binding of the fusion protein Clu-Strep to the Strep-Tactin column was too weak for purification and the method was then optimized for purification without any affinity tag by cation exchange chromatography followed by size exclusion chromatography.

2

Centrifuge culture and keep conditioned medium.

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3

Dialyze conditioned medium **Overnight** in **[M]20 Milimolar (mM)** Na acetate **pH5.0** (volume ratio <1:100).

4

If some precipitates are observed, remove by centrifugation.

Cation exchange chromatography

5

Load dialyzed conditioned medium into a HiTrap SP XL cation exchange column previously equilibrated with **[M]20 Milimolar (mM)** Na acetate **pH5.0**. Wash with **[M]20 Milimolar (mM)** Na acetate **pH5.0**.

6

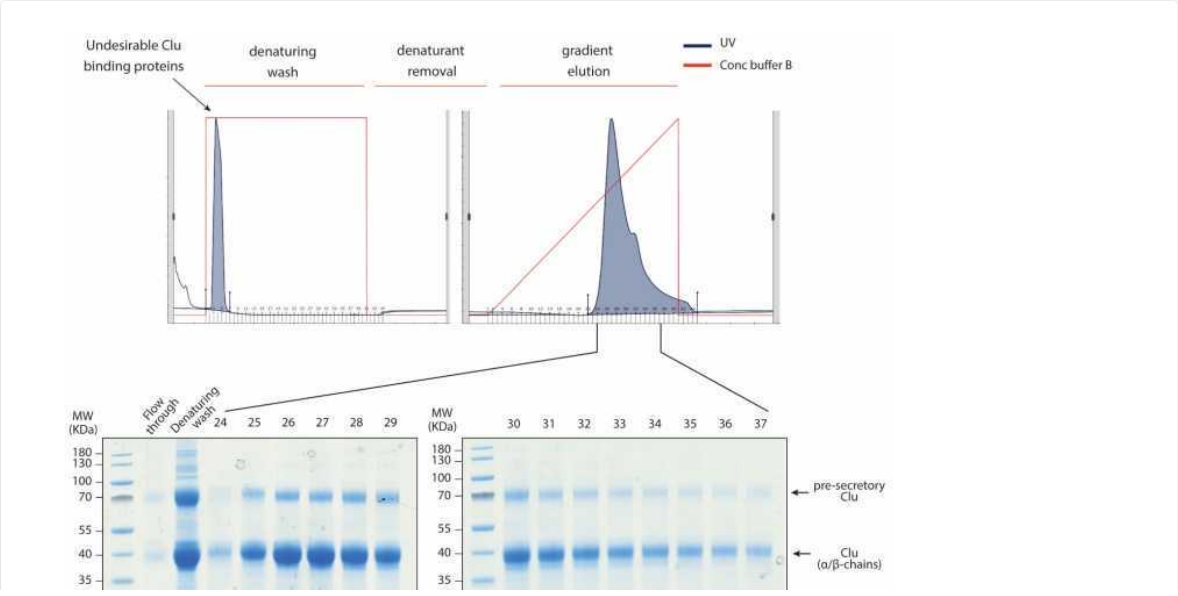
Wash the column with 10 column volumes (CV) of denaturing buffer (**[M]20 Milimolar (mM)** Na acetate **pH5.0**, **[M]6 Molarity (M)** urea) to remove undesired proteins bound to Clu.

7

Wash the column with 5 CVs **[M]20 Milimolar (mM)** Na acetate **pH5.0**.

8 Elute Clu with a **[M]0 Milimolar (mM)** - **[M]500 Milimolar (mM)** linear NaCl gradient in **[M]20 Milimolar (mM)** Na acetate **pH5.0**.

9 Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.



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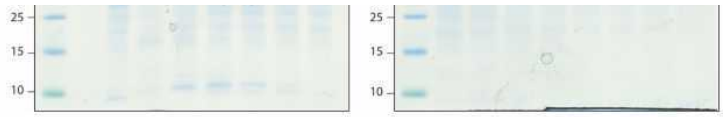


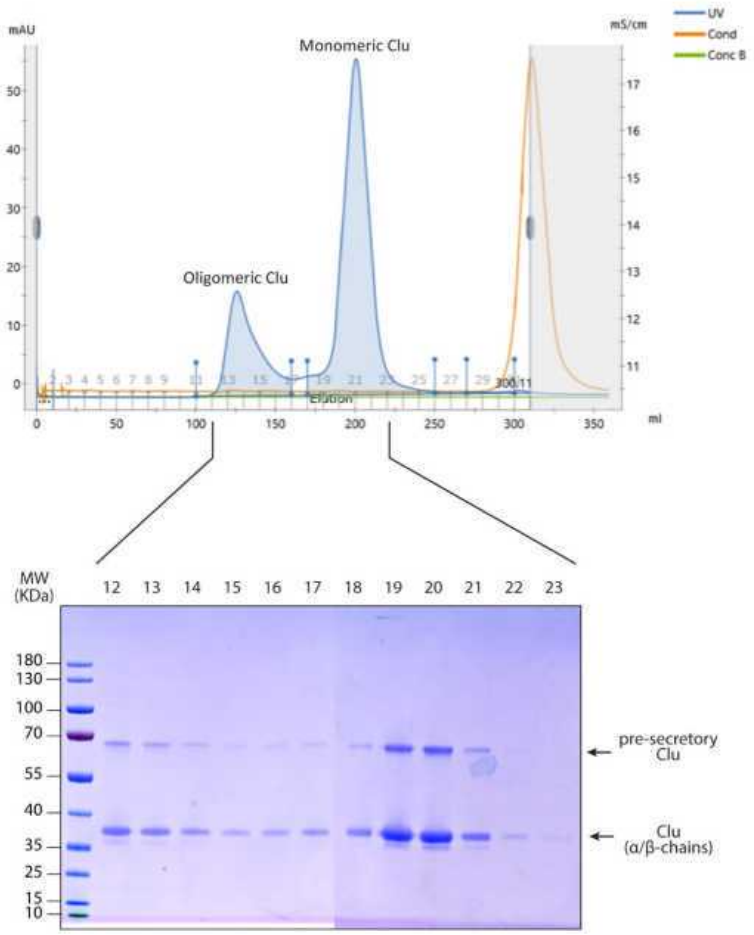
Note: Denaturing wash can be omitted if not many contaminants are observed in the conditioned media. A small percentage of Clu in the conditioned media is not cleaved at the furin-like protease cleavage site (pre-secretory Clu), probably due to its high expression level. Clus is highly glycosylated and migrates in the denaturing SDS-PAGE gel at around 40 kDa (α and β chains, not resolved) or around 70 kDa (pre-secretory, uncleaved Clu)

Size exclusion chromatography

10 Load Clu-containing fractions into a Superdex-200 previously equilibrated [M]**20 Milimolar (mM)** Na acetate pH**5.0**, [M]**100 Milimolar (mM)** NaCl, [M]**1 Milimolar (mM)** EDTA.

11 Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.

Clu oligomers are in equilibrium with monomeric Clu so all peaks containing Clu can be pooled. Oligomeric state of Clu is pH dependent. At pH 5.0 mainly monomeric Clu is eluted.



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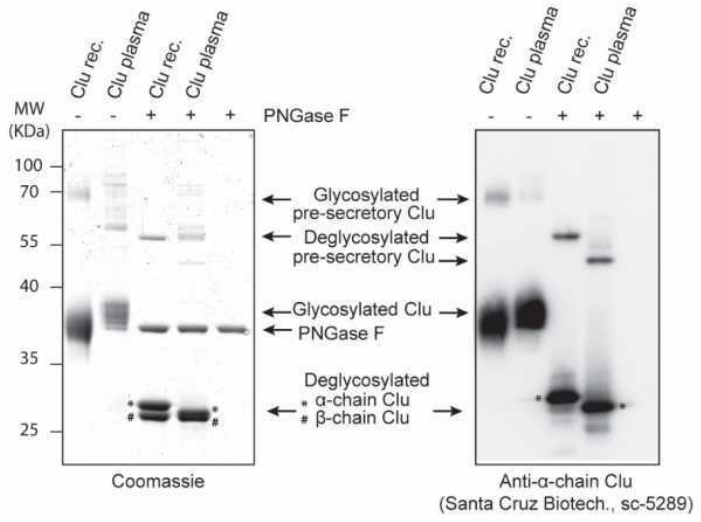
12 Concentrate Clu-containing fractions using a Vivaspin ultracentrifugation unit 10,000 MWCO or similar until reach desired concentration.

13 Aliquot and flash-freeze purified Clu in liquid nitrogen for storage at **-80 °C** .

Note: Approximate yield: from 250 ml of conditioned media around 12 mg of pure Clu are obtained. Clu purified from HEK293E cells present a comparable glycosylation pattern as Clu purified from plasma (Biovendor R&D, RD172034100). In order to obtain sharp bands of the α and β Clu chains in the SDS-PAGE, deglycosylation can be performed with PNGase F.



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