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

Clusterin purification from HEK293E cells

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ABSTRACT

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

ATTACHMENTS

[dtisbiezx.pdf](#)

MATERIALS

Buffers:

Binding buffer: 20 millimolar (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

Created: Jun 16, 2021

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PROTOCOL integer ID:
50828

Keywords: Clusterin
purification, HEK293E cells,
ASAPCRN

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

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.

- 3 Dialyze conditioned medium Overnight in 20 millimolar (mM) Na acetate 5.0 (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 20 millimolar (mM) Na acetate pH 5.0 . Wash with 20 millimolar (mM) Na acetate pH 5.0 .
- 6 Wash the column with 10 column volumes (CV) of denaturing buffer (20 millimolar (mM) Na acetate pH 5.0 , 6 Molarity (M) urea) to remove undesired proteins bound to Clu.
- 7 Wash the column with 5 CVs 20 millimolar (mM) Na acetate pH 5.0 .
- 8 Elute Clu with a 0 millimolar (mM) – 500 millimolar (mM) linear NaCl gradient in 20 millimolar (mM) Na acetate pH 5.0 .
- 9 Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.

Note

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
 [1M] 20 millimolar (mM) Na acetate $\text{pH } 5.0$, [1M] 100 millimolar (mM) NaCl,
 [1M] 1 millimolar (mM) EDTA.
- 11 Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.

Note

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.

- 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

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.