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Very low-density lipoprotein receptor (VLDLR)-C-tag purification from HEK293E cells

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

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Abstract

This protocol details how to purify recombinant Very low-density lipoprotein receptor (VLDLR)-C-tag protein from HEK293E cells.

Attachments

DOCX

VLDLR-C-tag purifica...

317KB

Materials

Buffers

- **Binding buffer:**

	A	B
	Tris-HCl pH 7.2	20 mM
	NaCl	100 mM
	CaCl2	0.5 mM

- **Elution buffer:**

	A	B
	Tris-HCl pH 7.0	20 mM
	MgCl2	2 M
	CaCl2	2 mM

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

CaptureSelect™ tPA Affinity Matrix **Thermo Fisher** **Catalog #**2943430005

Amersham NAP-25 Columns **Cytiva** **Catalog #**17-0852-01

VLDLR-C-tag expression

1 Express VLDLR-C-tag in HEK293E cells cultured in FreeStyle 293 Expression Medium for 96:00:00 4d

2 Centrifuge culture and keep conditioned medium.

3 Dialyze 300 mL conditioned medium Overnight against 10 L Binding buffer.

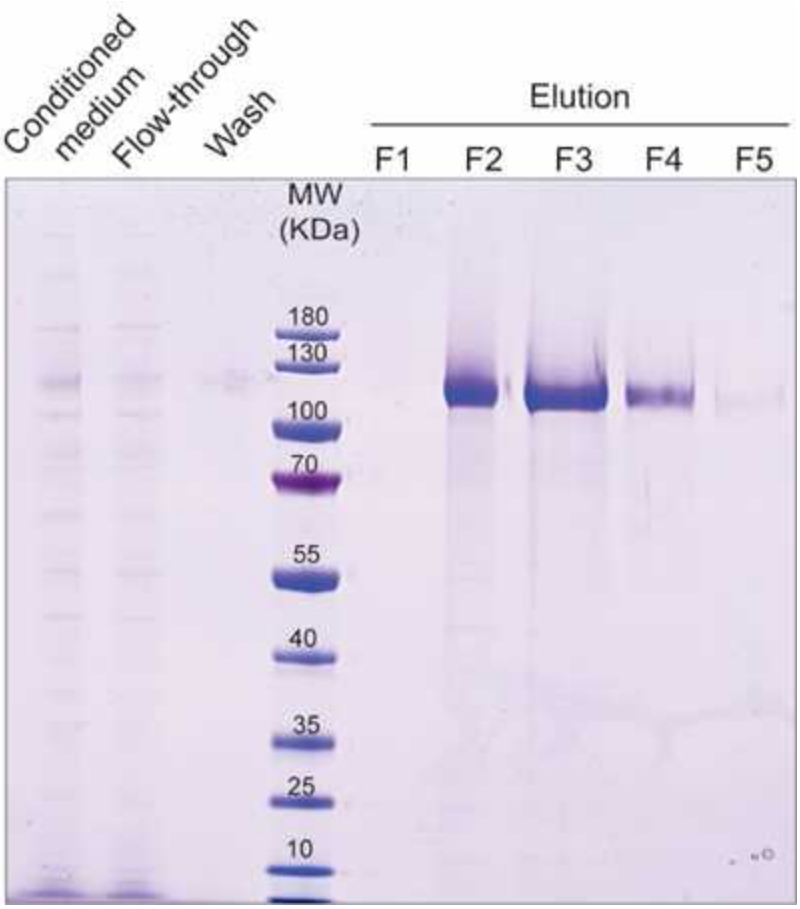
CaptureSelect C-tag affinity chromatography

4 Load dialyzed conditioned medium onto a CaptureSelect C-tag affinity column previously equilibrated with binding buffer (column volume, CV: 4 mL slurry for 300 mL dialyzed media) by gravity flow at 4 °C .

5 Wash the column with 5 CV of Binding buffer.

6 Elute VLDLR-C-tag protein with 6× 1 mL of Elution buffer. Collect fractions of 1 mL .

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



8 Pool fractions containing VLDLR-C-tag protein.

9 Exchange protein buffer to Binding buffer with a NAP-25 desalting column previously equilibrated with Binding buffer.

10 Collect the fractions containing protein, concentrate by ultrafiltration to >1 mg mL-1, aliquot and flash-freeze purified VLDLR-C-tag in liquid nitrogen for storage at -70 °C .

Note

Approximate yield: From 300 ml of conditioned media around 0.6 mg of pure VLDLR-C-tag were obtained. Yield can be significantly increased if the VLDLR chaperone, RAP is co-overexpressed during protein production.