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Ion-exchange purification of fucoidans

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

Fucoidans are a diverse class of sulfated polysaccharides integral to the cell wall of brown algae and due to their various bioactivities, they are potential drugs. Standardized work with fucoidans is required for structure-function studies, but remains challenging since available fucoidan preparations are often contaminated with other algal compounds. Additionally, fucoidans are structurally diverse depending on species and season, urging the need for standardized purification protocols. Here, we use ion-exchange chromatography to purify different fucoidans and found a high structural diversity between fucoidans. Ion-exchange chromatography efficiently removes the polysaccharides alginate and laminarin and other contaminants such as proteins and phlorotannins across a broad range of fucoidans from major brown algal orders including *Ectocarpales*, *Laminarinales* and *Fucales*. By monomer composition, linkage analysis and NMR characterization, we identified glucuronic acid and O-acetylation as new structural features of certain fucoidans and provide a novel structure of fucoidan from *Durvillaea potatorum* with a-1,3 linked fucose backbone and b-1,6 and b-1,3 galactose branches. This study emphasizes the use of standardized ion-exchange chromatography to obtain defined fucoidans for subsequent molecular studies.

PROTOCOL CITATION

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KEYWORDS

Fucoidan, Ion-exchange chromatography, Brown algae, Sulfated polysaccharides

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MATERIALS

NAME	CATALOG #	VENDOR
Sodium Chloride	PubChem CID: 5234	
Tris (Tris Base)	T-400	Gold Biotechnology
Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane, 1000mL, 0.2μm pore, 33mm neck	597-3320	Thermo Fisher
ANX Sepharose 4 Fast Flow	17128701	Ge Life Sciences
XK 50/20 Column	28988952	Ge Life Sciences
Fucus serratus fucoidan	YF157167	Carbosynth
Ecklonia maxima fucoidan	YF157166	Carbosynth
Sargassum fusiforme fucoidan	YF157167	Carbosynth
Macrocystis pyrifera fucoidan	YF145109	Carbosynth

NAME	CATALOG #	VENDOR
Cladosiphon okamuranus fucoidan	YF146834	Carbosynth
Durvillaea potatorum fucoidan	YF157165	Carbosynth
Lessonia nigrescens fucoidan	YF146833	Carbosynth

Simple chromatography setup 1h

1 Prepare a simple chromatography system consisting of:
1. MasterFlex L/S peristaltic pump
2. XK50/250 column (GE healthcare)
3. Suitable tubings and connectors
4. Slurry 200 mL of the ANX and pack it into the column
5. Compress the resin by applying a gentle flow (~5 mL/min)


IEX_setup.png

Buffer preparation 1h

2
1. ~2 liters of double de-ionized water (ddH_2O)
2. ~2 liters of 50 mM Tris-HCl pH=7.5 in ddH_2O (Buffer A)
3. ~1 liters of 50 mM Tris-HCl pH=7.5 in ddH_2O with 0.5 M NaCl (Buffer B)
4. ~1 liters of 50 mM Tris-HCl pH=7.5 in ddH_2O with 5 M NaCl (Buffer C)
5. ~1 liters of250 mM NaOH in ddH_2O

3 Filter all buffers through a 0.2 µm bottle top filter to remove dust

3.1 Rinse filters with ddH_2O and dry for reuse

Prepare polysaccharide solution 4h

4 Mix 0.5 g fucoidan in 500 mL of 50 mM Tris pH 7.5 in ddH_2O

5 Use a magnetic stirrer and heat ~50-60°C for ~1h or until fucoidans dissolved

6 Transfer solution into centrifuge vials and spin down at **4500 x g , for 30'**

7 Carefully decant supernatant into new bottle, avoid transfering solids

Chromatography 20m

8 Set the flow of the pump to 40-50 mL/min

9 Equilibrate the column with 3 CV Buffer A

10 Apply fucoidan in a semi-batch mode. For this, connect the outlet tube to your input reservoir to circulate the solution at least 3x over the column.

11 Wash with 1 CV Buffer A

12 Wash with 3 CV Buffer B

13 Elute with 0.5 CV Buffer C

13.1 ⚠

Keep in mind the void volumn of your column (~200 mL). After switching to Buffer C, you need to wait one CV. The high salt concetration of Buffer C results in visible schlieren in a low salt buffer.

13.2 Collect the first 100 mL of eluted Buffer C

13.3 Proceed with the eluted fraction to the dialysis

14 Wash column

14.1 2 CV Buffer C

14.2 2 CV ddH_2O

14.3 2 CV NaOH

14.4 2 CV ddH_2O or until pH is neutral

14.5 For long-term storage of the column, use 20% Ethanol in ddH_20

Post processing 2h

15 Transfer the eluted polysaccharide fraction into a 1 kDa dialysis tubing

16 Dialyse against ddH2O and change the ddH2O several times

17 Transfer the liquid into a new vial

18 Freeze-dry the sample