

Chemical Affinity-Based Isolation of Extracellular Vesicles from Biofluids for Proteomics and Phosphoproteomics Analysis

 Yi-Kai Liu¹, Zhuojun Luo¹, Anton Iliuk^{1,2}, Weiguo Andy Tao^{1,2,3,4}
¹Department of Biochemistry, Purdue University ²Tymora Analytical Operations ³Department of Chemistry, Purdue University ⁴Purdue Institute for Cancer Research, Purdue University

Corresponding Authors

Anton Iliuk
 anton.iliuk@tymora-analytical.com

Weiguo Andy Tao
 taow@purdue.edu

Citation

Liu, Y.K., Luo, Z., Iliuk, A., Tao, W.A. Chemical Affinity-Based Isolation of Extracellular Vesicles from Biofluids for Proteomics and Phosphoproteomics Analysis. *J. Vis. Exp.* (200), e65844, doi:10.3791/65844 (2023).

Date Published

October 27, 2023

DOI

10.3791/65844

URL

jove.com/video/65844

Materials

Name	Company	Catalog Number	Comments
1.5 mL microcentrifuge tube	Life Science Products	M-1700C-LB	
1.5 mL tube magnetic separator rack	Sergi Lab Supplies	1005	
15 mL conical centrifuge tube	Corning	352097	
15 mL tube magnetic separator rack	Sergi Lab Supplies	1002	
Anti-rabbit IgG, HRP-linked Antibody	Cell Signaling Technology	7074P2	
Benchtop incubated shaker	Bioer	DIS-87999-3367802	Bioer Thermocell Mixing Block MB-101
CD9 (D3H4P) Rabbit mAb	Cell Signaling Technology	13403S	
Chloroacetamide	Sigma -Aldrich	C0267-100G	Used for alkylation of reduced sulfide groups. Freshly prepare 400 mM in water as stock solution.
Ethyl acetate	Fisher Scientific	E145-4	Precipitates detergents
Evosep One	Evosep		Liquid chromatography system
Evotips	Evosep	EV2013	Sample loading for Evosep One system
EVtrap	Tymora Analytical		Functionalized magnetic beads, loading buffer, and washing buffer
Immobilon-FL PVDF Membrane	Sigma -Aldrich	IPFL00010	Blotting membrane
NuPAGE 4-12% Bis-Tris Gel	Invitrogen	NP0322BOX	Invitrogen NuPAGE 4 to 12%, Bis-Tris, 1.0 mm, Mini Protein Gel, 12-well
NuPAGE LDS Sample Buffer (4X)	Invitrogen	NP0007	
PBS	ThermoFisher	10010023	
Pepsep C18 15 x 75 x 1.9	Bruker	1893473	Separation column
Phosphatase Inhibitor Cocktail 2	Sigma -Aldrich	P5726-5ML	100X, Phosphatase inhibitor.
Phosphatase Inhibitor Cocktail 3	Sigma -Aldrich	P0044-1ML	100X, Phosphatase inhibitor.
Pierce BCA Protein Assay Kit	ThermoFisher	23225	

Pierce ECL Western Blotting Substrate	ThermoFisher	32106	HRP substrate
PolyMAC phosphopeptide enrichment kit	Tymora Analytical		Polymer-based metal ion affinity capture (PolyMAC) for phosphopeptide enrichment
Sodium deoxycholate	Sigma -Aldrich	D6750-10G	Detergent for lysis buffer. Prepare 120 mM in water as stock solution.
Sodium lauroyl sarcosinate	Sigma -Aldrich	L9150-50G	Detergent for lysis buffer. Prepare 120 mM in water as stock solution.
timsTOF HT	Bruker		Trapped ion-mobility time-of-flight mass spectrometry
TopTip C-18 (10-200 μ L) tips	Glygen	TT2C18.96	Desalting method
Triethylamine	Sigma -Aldrich	471283-100ML	For EV elution.
Triethylammonium bicarbonate buffer	Sigma -Aldrich	T7408-100ML	1 M
Trifluoroacetic acid	Sigma -Aldrich	302031-100ML	
Tris-(2-carboxyethyl)phosphine hydrochloride	Sigma -Aldrich	C4706	Used for reduction of disulfide bonds. Prepare 200 mM in water as stock solution. Aliquot the stock solution into small volume and store it in at -20°C (avoid multiple freeze-thaw cycles).
Trypsin/Lys-C MIX	ThermoFisher	PIA41007	