



# Discover Exosome Proteins

Exosomes are nano-sized vesicles secreted by every cell type in vivo and in vitro. These extracellular vesicles transport specific populations of proteins, lipids, RNAs during intercellular communication. The contents of exosomes vary depending upon the source cell and can serve as a “molecular fingerprint” of disease states. SBI’s exosome Mass Spec Custom Service provides researchers with comprehensive, expert proteomic sample preparation and analysis to identify protein biomarkers from exosomes using LC/MS/MS Mass Spectroscopy. The service is a complete start-to-finish solution; just provide biofluid samples (i.e. serum, plasma, culture media, spinal fluid, urine, etc.) and SBI does the rest. Sample preparation requirements and the service workflow details are listed below.

## Input Sample Requirements

Biofluid	Volume
Serum	500µl - 1ml
Plasma	500µl - 1ml
Cell Media	5ml - 10ml
Urine	5ml - 10ml
Spinal Fluid	5ml - 10ml
Ascites Fluid	500µl - 1ml
Other	Inquire

## Exosome MS Data Deliverables

- All raw MS spectra data are available
- Pre-analyzed data in Scaffold format provided
- Excel file with proteins identified and quantified
- Turnaround time is 4 weeks start to finish

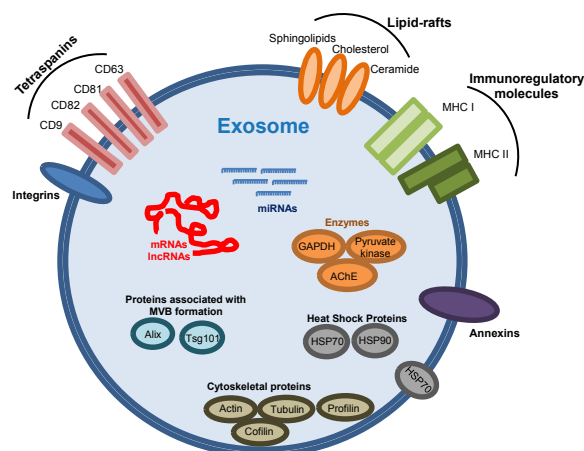


**FIND OUT MORE:**

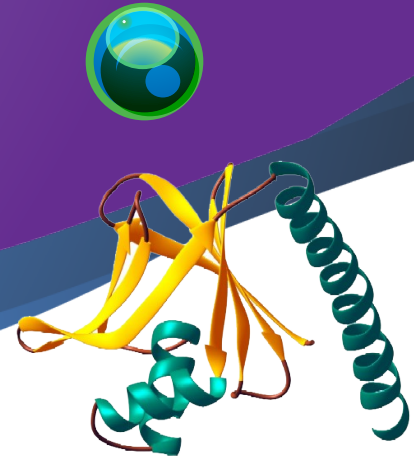
[www.systembio.com/xprotein](http://www.systembio.com/xprotein)

## Building Exosome Peptide Libraries

- ▶ Exosomes isolated using ExoQuick®
- ▶ Peptide libraries made from total or surface shaving methods (your choice)
- ▶ All libraries prepared using standard trypsin cleavage for MASCOT compatibility
- ▶ Proxeon EASY-nLC 1000 HPLC system interfaced to a ThermoFisher Q Exactive mass spectrometer used for analysis



# Full Data Analysis



## Sample Human Serum Exosome Data

Exosomes were isolated from 500ul of control human serum using ExoQuick. The exosome pellet was processed and complete peptide libraries were then analyzed by nano LC/MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher Q Exactive. Peptides were loaded on a trapping column and eluted over a 75um analytical column at 350nL/min using a 2hr reverse phase gradient; both columns were packed with Jupiter Proteo resin (Phenomenex). The injection volume was 30uL. The mass spectrometer was operated in data-dependent mode, with the Orbitrap operating at 60,000 FWHM and 17,500 FWHM for MS and MS/MS respectively. The fifteen most abundant ions were selected for MS/MS and data analyzed using MASCOT databases and Scaffold software. The table to the right shows some example serum exosome protein MS/MS data with typical exosome proteins highlighted in yellow.

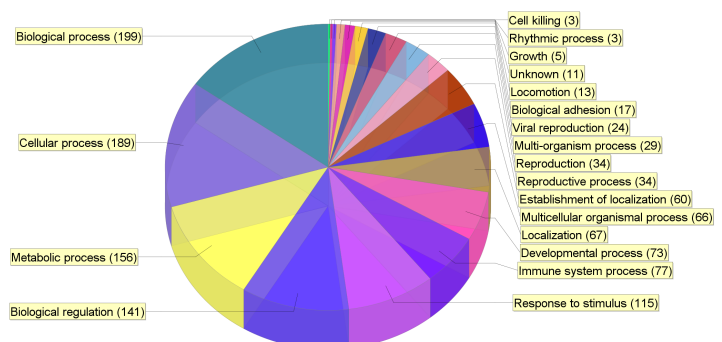
Scaffold software data viewer makes it easy to mine your data quickly (included).

- Robust statistical validation
- Label free quantitation
- Protein homology analysis
- Publication-ready graphics

**FIND OUT MORE:**  
[www.systembio.com/xprotein](http://www.systembio.com/xprotein)

Identified Proteins	Accession Number	MW (kD)	Complete
Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=1	sp P04264 K2C1_HUMAN	66 kDa	32
Tax. Id=9606 Gene_Symbol=KRT10 Keratin, type I cytoskeletal 10	IPI:CON_00009865.2 SWISS-PROT	60 kDa	27
Tax. Id=9606 Gene_Symbol=KRT6A Keratin, type II cytoskeletal 6A	IPI:CON_00300725.7 SWISS-PROT	60 kDa	8
Tax. Id=9606 Gene_Symbol=KRT5 Keratin, type II cytoskeletal 5	IPI:CON_00009867.3 SWISS-PROT	62 kDa	8
Prohibitin-2 OS=Homo sapiens GN=PHB2 PE=1 SV=2	sp Q99623 PHB2_HUMAN	33 kDa	11
Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2	sp P35908 K2E2_HUMAN	65 kDa	21
Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=HVA1 PE=1 SV=1	sp P21796 VDAC1_HUMAN	31 kDa	6
Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1	sp P63261 ACTG_HUMAN	42 kDa	61
Tax. Id=9606 Gene_Symbol=KRT14 Keratin, type I cytoskeletal 14	IPI:CON_00384444.5 SWISS-PROT	52 kDa	7
Sideroflexin-1 OS=Homo sapiens GN=SFKN1 PE=1 SV=4	sp Q9H9B4 SFKN1_HUMAN	36 kDa	3
Prohibitin OS=Homo sapiens GN=PHB PE=1 SV=1	sp P35232 PHB_HUMAN	30 kDa	9
Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A	sp P08107 HSP71_HUMAN	70 kDa	82
Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=	sp P04406 G3P_HUMAN	36 kDa	38
ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7	sp P05141 ADT2_HUMAN	33 kDa	11
Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1	sp P08238 HS90B_HUMAN	83 kDa	61
60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSP60	sp P10809 CH60_HUMAN	61 kDa	28
Tax. Id=9606 Gene_Symbol=KRT9 Keratin, type I cytoskeletal 9	IPI:CON_00019359.3 SWISS-PROT	62 kDa	7
Annexin A5 OS=Homo sapiens GN=ANXA5 PE=1 SV=2	sp P08758 ANXA5_HUMAN	36 kDa	17
CD81 antigen OS=Homo sapiens GN=CD81 PE=1 SV=1	sp P06033 CD81_HUMAN	26 kDa	9
Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=	sp P11279 LAMP1_HUMAN	45 kDa	2
CD9 antigen OS=Homo sapiens GN=CD9 PE=1 SV=4	sp P21926 CD9_HUMAN	25 kDa	5
Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1	sp P07900 HS90A_HUMAN	85 kDa	58
Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3	sp P01023 A2MG_HUMAN	163 kDa	34
Voltage-dependent anion-selective channel protein 3 OS=Homo sapiens GN=	sp Q9Y277 VDAC3_HUMAN	31 kDa	4
ADP/ATP translocase 3 OS=Homo sapiens GN=SLC25A6 PE=1 SV=4	sp P12236 ADT3_HUMAN	33 kDa	11
Poly(rC)-binding protein 2 OS=Homo sapiens GN=PCBP2 PE=1 SV=1	sp Q15366 PCBP2_HUMAN	39 kDa	4
Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	sp P01024 C3_HUMAN	187 kDa	31
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 5	sp P04843 RPN1_HUMAN	69 kDa	9
Phosphate carrier protein, mitochondrial OS=Homo sapiens GN=SLC25A3	sp Q00325 MPCP_HUMAN	40 kDa	3
Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2	sp P07437 TUBB5_HUMAN	50 kDa	50
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8	sp P11142 HSP7C_HUMAN	71 kDa	42
Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3	sp Q06014 H2BK_HUMAN (+7)	14 kDa	27
Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2	sp P07355 ANXA2_HUMAN	39 kDa	9
Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=	sp P05023 AT1A1_HUMAN	113 kDa	7
Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1	sp P68363 TBA1B_HUMAN	50 kDa	40

## Visualize and Validate Complex Exosome MS/MS Proteomics Experiments



Compare proteomic profiles across patient samples to discover novel biomarkers.