

Materials List for:

# Glycoproteomics of the Extracellular Matrix: A Method for Intact Glycopeptide Analysis Using Mass Spectrometry

Javier Barallobre-Barreiro<sup>1</sup>, Ferheen Baig<sup>1</sup>, Marika Fava<sup>1</sup>, Xiaoke Yin<sup>1</sup>, Manuel Mayr<sup>1</sup>

<sup>1</sup>King's British Heart Foundation Centre, King's College London

Correspondence to: Manuel Mayr at [manuel.mayr@kcl.ac.uk](mailto:manuel.mayr@kcl.ac.uk)

URL: <https://www.jove.com/video/55674>

DOI: [doi:10.3791/55674](https://doi.org/10.3791/55674)

## Materials

Name	Company	Catalog Number	Comments
<b>A. Chemicals</b>			
Acetonitrile, MS-grade (ACN, C <sub>2</sub> H <sub>3</sub> N)	Thermo Scientific	51101	5.2-5.8, 6.2, 7.11, Supp 2, 3, 4
Cocktail of proteinase inhibitors	Sigma-Aldrich	P8340	1.3, 1.4.1, 1.5.1, 1.6.1
Disodium phosphate (Na <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> )	Sigma-Aldrich	S7907	3.1
Dithiothreitol (DTT, C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> S <sub>2</sub> )	Sigma-Aldrich	D0632	4.3
Ethylenediaminetetraacetic acid (EDTA, C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>8</sub> )	Sigma-Aldrich	E9884	1.3, 1.4.1, 1.5.1, 1.6.1
Ethanol (C <sub>2</sub> H <sub>6</sub> O)	VWR	437433T	2.2.1
Guanidine hydrochloride (GuHCl, CH <sub>6</sub> ClN <sub>3</sub> )	Sigma-Aldrich	G3272	1.6.1
Glycerol (C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> )	Acros organics	158920025	Suppl 1.1
H <sub>2</sub> O LC-MS Cromasolv	Sigma-Aldrich	39253-1L-R	Throughout the protocol
H <sub>2</sub> <sup>18</sup> O	Taiyo Nippon Sanso	FO3-0027	3.5
Hydroxylamine (HA, H <sub>3</sub> NO)	Sigma-Aldrich	467804	6.4
Iodoacetamide (IAA, C <sub>2</sub> H <sub>4</sub> INO)	Sigma-Aldrich	A3221	4.4
Phosphate-buffered Saline (PBS), 10x	Lonza	51226	1.3
Sodium acetate (C <sub>2</sub> H <sub>3</sub> NaO <sub>2</sub> )	Sigma-Aldrich	S7545	1.6.1
Sodium chloride (NaCl)	Sigma-Aldrich	S9888	1.4.1, 3.2
Sodium dodecyl sulfate (SDS, NaC <sub>12</sub> H <sub>25</sub> SO <sub>4</sub> )	Sigma-Aldrich	466143	1.5.1
Triethylammonium bicarbonate (TEAB, C <sub>7</sub> H <sub>17</sub> NO <sub>3</sub> )	Sigma-Aldrich	11268	4.7, 6.1
Trifluoroacetic acid (TFA, C <sub>2</sub> HF <sub>3</sub> O <sub>2</sub> )	Sigma-Aldrich	T62200	4.8, 5.2-5.8, 7.11, Supp 2, 3
Thiourea (CH <sub>4</sub> N <sub>2</sub> S)	Sigma-Aldrich	T8656	4.2
Tris-hydrochloride (Tris-HCl, NH <sub>11</sub> C <sub>4</sub> O <sub>3</sub> [HCl])	Sigma-Aldrich	T3253	1.4.1, Suppl 1.
Urea (CH <sub>4</sub> N <sub>2</sub> O)	Sigma-Aldrich	U1250	4.2
<b>Name</b>			
<b>Company</b>			
<b>Catalog Number</b>			
<b>Comments</b>			
<b>B. Enzymes</b>			
α2-3,6,8,9-Neuraminidase (Sialidase)	EDM Millipore	362280 (KP0012)	3.1
β1,4-Galactosidase	EDM Millipore	362280 (KP0004)	3.1
β-N-Acetylglucosaminidase	EDM Millipore	362280 (KP0013)	3.1

Chondroitinase ABC	Sigma-Aldrich	C3667	3.1
Endo- $\alpha$ -N-acetylgalactosaminidase (O-glycosidase)	EDM Millipore	362280 (KP0011)	3.1
Heparinase II	Sigma-Aldrich	H6512	3.1
Keratanase	Sigma-Aldrich	G6920	3.1
PNGase-F (N-Glycosidase F)	EDM Millipore	362280 (KP0001)	3.5
Trypsin	Thermo Scientific	90057	4.7
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>C. Reagent kits</b>			
30 kDa MWCO spin filters	Amicon, Millipore	10256744	5.9, Suppl 2
Macro SpinColumn C-18, 96-Well Plate	Harvard Apparatus	74-5657	5.1
NuPAGE Novex BisTris Acrylamide Gels	Thermo-Scientific	NP0322PK2	Suppl 1
Pierce BCA Protein Assay Kit	Thermo Scientific	23227	2.1.3
ProteoExtract Glycopeptide Enrichment Kit	Merk Millipore	72103	7
Tandem mass tag 0 (TMT <sup>0</sup> )	Thermo Scientific	900067	6.2, 6.3
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>D. Equipment and software</b>			
Acclaim PepMap100 C18 Trap, 5 mm x 300 $\mu$ m, 5 $\mu$ m, 100 Å	Thermo Scientific	160454	Suppl 3, 4
Acclaim PepMap100 C18, 50 cm x 75 $\mu$ m, 3 $\mu$ m, 100 Å	Thermo Scientific	164570	Suppl 3
Byonic Search Engine	Protein Metrics	Version 2.9.30	Suppl 5
Dionex UltiMate 3000 RSLCnano	Thermo Scientific	n/a	Suppl 3, 4
EASY-Spray Ion Source	Thermo Scientific	ES081	Suppl 4
EASY-Spray PepMap RSLC C18, 50 cm x 75 $\mu$ m, 2 $\mu$ m, 100 Å	Thermo Scientific	ES803	Suppl 4
Mascot Search Engine	Matrix Science	Version 2.3.01	Suppl 3
Orbitrap Fusion Lumos Tribrid Mass Spectrometer	Thermo Scientific	IQLAAEGAAPFADMBHQ	Suppl 4
Proteome Discoverer Software	Thermo Scientific	Version 2.1.1.21	Suppl 3, 5
Picoview Nanospray Source	New Objective	550	Suppl 3
Q Exactive HF Mass Spectrometer	Thermo Scientific	IQLAAEGAAPFALGMBFZ	Suppl 3
Savant SpeedVac Concentrator	Thermo Scientific	SPD131DDA	2.2.2, 3.4, 4.6, 5.7, 6.5, 7.11
Scaffold Software	Proteome Software	Version 4.3.2	Suppl 3