

Materials List for

2D-HELMS MS Seq: A General LC-MS-Based Method for Direct and de novo Sequencing of RNA Mixtures with Different Nucleotide Modifications

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Materials

Name	Company	Catalog Number	Comments
5' DNA Adenylation kit	New England Biolabs	E2610S	50uM concentration
6550 Q-TOF mass spectrometer	Agilent Technologies	5991-2116EN	Coupled to a 1290 Infinity LC system
A(5')pp(5')Cp-TEG-biotin-3'	ChemGenes	91718	HPLC purified
ATPγS	Sigma-Aldrich	11162306001	Lithium salt
Bicine	Sigma-Aldrich	B8660	BioXtra, ≥99% (titration)
Biotin maleimide	Vector Laboratories	SP-1501	Long arm
C18 column	Waters	186003532	50 mm × 2.1 mm Xbridge C18 column with a particle size of 1.7 μm
Centrifugal Vacuum Concentrator	Labconco	Refrig 115v/60hz 7310022	Labconco CentriVap
ChemBioDraw	PerkinElmer	ChemDraw Prime	Generate a chemical structure and property data of structures & fragments
CMC (N-cyclohexyl-N'-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulfonate)	Sigma-Aldrich	2491-17-0	95% Purify
Cyanine3 maleimide (Cy3)	Lumiprobe	11080	Water insoluble
DEPC-treated water	Thermo Fisher Scientific	AM9906	Autoclaved, certified nuclease-free
Diisopropylamine (DIPA)	Thermo Fisher Scientific	108-18-9	99% Alfa Aesar
DMSO	Sigma-Aldrich	276855	Anhydrous dimethyl sulfoxide, 99.9%
EDTA	Sigma-Aldrich	E6758	Anhydrous, crystalline, BioReagent, suitable for cell culture
Formic acid	Merck	64-18-6	98-100%, ACS reagent, Ph Eur
Hexafluoro-2-propanol (HFIP)	Thermo Fisher Scientific	920-66-1	99% Acros Organics
LC-MS sample vials	Thermo Fisher Scientific	C4000-11	Plastic screw thread vials
LC-MS vial caps	Thermo Fisher Scientific	C5000-54A	Autosampler vial screw thread caps
Na ₂ CO ₃ buffer	Sigma-Aldrich	88975	BioUltra, >0.1 M Na ₂ CO ₃ , >0.2 M NaHCO ₃

Oligo Clean & Concentrator	Zymo Research	D4060	Spin column
OriginLab	OriginLab	OriginPro	Data analysis and graphing software
pCp-biotin	TriLink BioTechnologies	NU-1706-BIO	20 ul (1 mM)
RNA #1--#6	Integrated DNA Technologies	Custom RNA oligos	19nt-21nt single-stranded RNAs, used without further purification
Rocking platform shaker	VWR	Orbital Shaker Standard 1000	Speed Range 40 to 300 rpm
Streptavidin magnetic beads	Thermo Fisher Scientific	88816	Binding approx. 55ug biotinylated rabbit IgG per mg of beads
Sulfonated Cyanine3 maleimide	Lumiprobe	11380	Water soluble
T4 DNA ligase 1	New England Biolabs	M0202S	400 units/uL
T4 polynucleotide kinase	Sigma-Aldrich	T4PNK-RO	From phage T4 am N81 pse T1 infected Escherichia coli BB
Tris-HCl buffer	Sigma-Aldrich	T6455	Tris-HCl Buffer, pH 10, 10x, Antigen Retriever
Urea	Sigma-Aldrich	81871	Urea for synthesis. CAS No. 57-13-6, EC Number 200-315-5.