Peptide Synthesis Report

MOFFITT CANCER CENTER

PROTEOMICS

Project Title: MMP9 Internal standard

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Design and Synthesis:

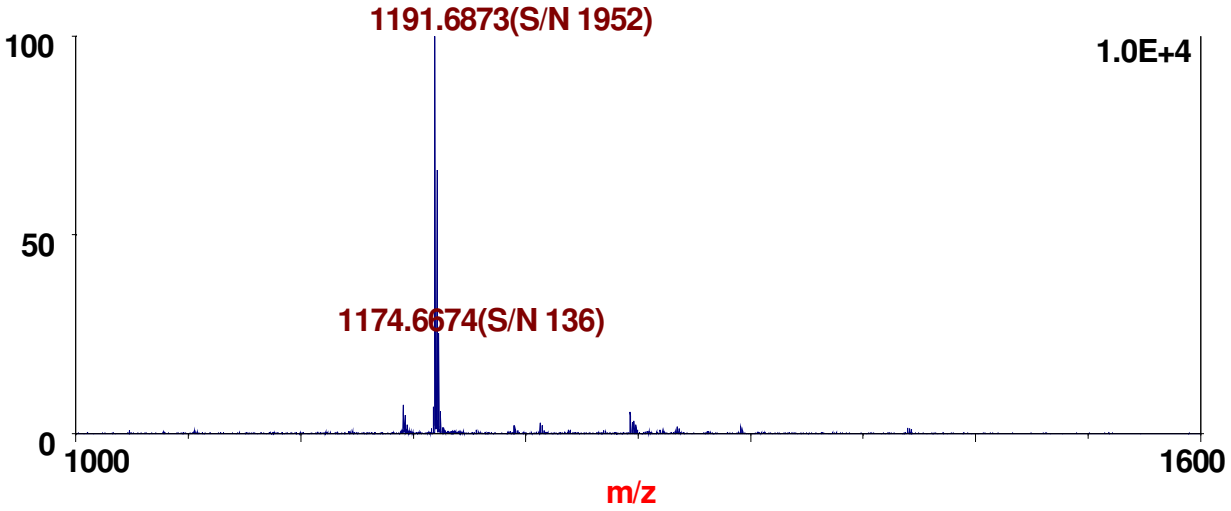
MPS#	Peptide Sequence	Residues	Modification	MWT Monoisotopic
p-307	QLAEEYLYR		Stable Isotope on L7 (U-13C6, 15N1)	1190.58738

Solid state peptide synthesis (Symphony, Protein Technologies, Tucson, AZ) is used to make standards at the 25 micromole scale using standard FMOC chemistry. Briefly, Wang resin (Novabiochem, Germany) serves as the support to initiate peptide formation. Activator solvents for the amino acid coupling consist of 150 mM N-Methylmorpholine in N-methylpyrrolidone (NMP) with 100 mM of O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU). Deprotection between couplings is achieved using 20% piperidine in NMP with 2% 1,8-diazabicyclo [5,4,0] undec-7-ene. Upon sequence completion, cleavage from the resin is performed using 95% TFA, 2.5% water, and 2.5% triisopropylsilane. Peptides are then precipitated in ice cold ethyl ether, washed twice, and resuspended in water prior to lyophilization.

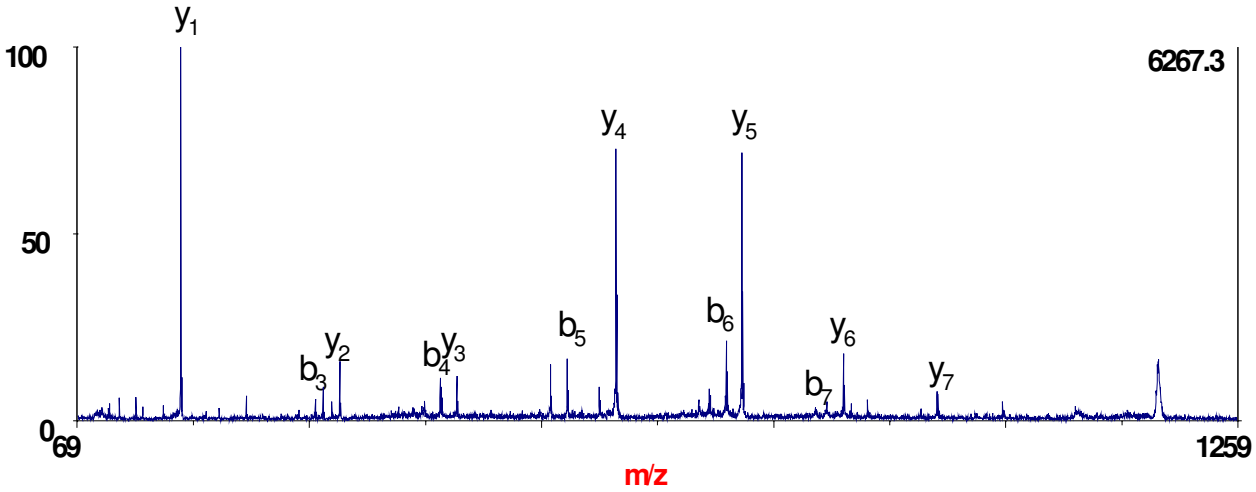
MALDI:

Purified peptides are analyzed with MALDI MS to verify purity and sequenced with MS/MS (4700, Applied Biosystems, Framingham, MA). Peptides in 2% ACN with 0.1% formic acid are mixed 1:1 with a-cyano-4-hydroxycinnamic acid (10 mg/ml) in 50% ACN and deposited in 1 microliter aliquots on the MALDI target.

MS Spectrum: ~93% pure



MS/MS spectrum:



Fragment Ion table with Identified peaks:

Seq	#	B	Y	# (+1)
Q	1	129.06645	1191.59521	9
L	2	242.15051	1063.53663	8
A	3	313.18762	950.45256	7
E	4	442.23022	879.41545	6
E	5	571.27281	750.37286	5
Y	6	734.33614	621.33027	4
L	7	854.42020	458.26694	3
Y	8	1017.48353	338.18287	2
R	9	1173.58464	175.11955	1