

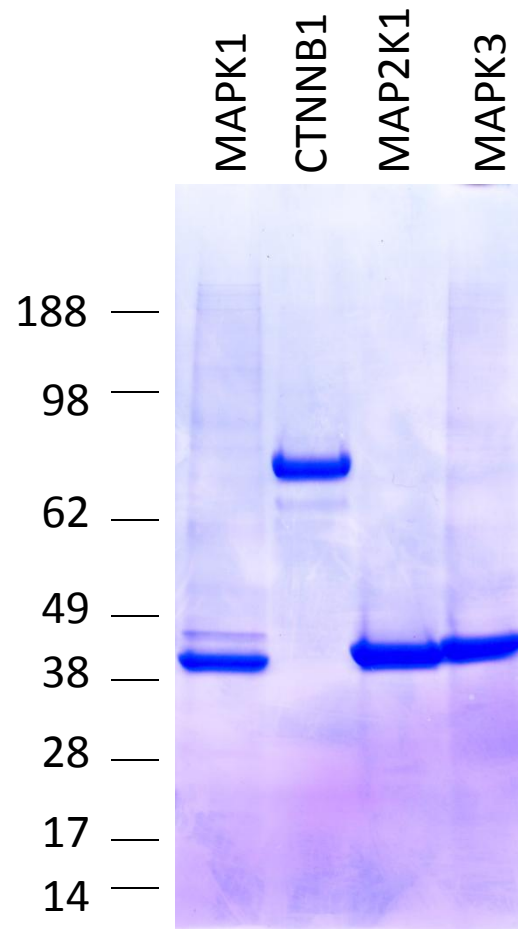
Incorporation efficiency of heavy isotopic amino acids into OriGene's Mass Spec Standards

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Introduction

- OriGene manufactured the heavy isotope labeled MAP2K1 and MAPK1 by transfecting HEK293T cells with over-expression clones.
- The cells were cultured in heavy isotope medium for over 10 generations and the proteins were isolated using anti-DDK affinity column.
- The heavy labeled proteins were sent to ISB for MS analysis and the incorporation efficiency were determined.
- For both samples, the incorporation efficiency is over 90%.

The C13 and N15 labeled recombinant proteins as Mass-Spec internal standard



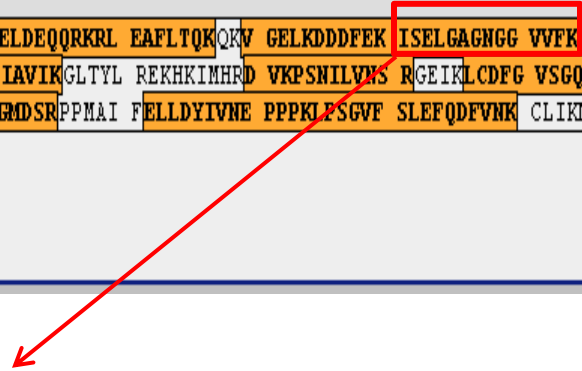
MAP2K1 and MAPK1 were chosen for Mass Spec analysis to determine heavy amino acid incorporation efficiency.

Peptides identified from MAP2K1 MS analysis

Protein: MAP2K1 (Cat#: [PH318460](#)) (Peptides detected by mass spec are highlighted in yellow)

Protein: IPI00219604

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>IPI00219604 IPI:IPI00219604.3|SWISS-PROT:Q02750 |TREMBL:A4QPA9;B4DFY5 |ENSEMBL:ENSP00000302486 |REFSEQ:NP_002746
|H-INV:HIT000192051|VEGA:OTTHUMP00000164252;OTTHUMP00000175899 Tax_Id=9606 Gene_Symbol=MAP2K1 Dual specificity mitogen-activated protein kinase kinase
1
MPK K K P T P I Q L N P A P D G S A V N G T S S A E T N L E A L Q K K L E E L E L D E Q Q R K R L E A F L T Q K Q K V G E L K D D D F E K I S E L G A G N G G V V F K V S H K P S G L V M A R K L I H L E I K P A I R N Q I I R E L Q V L H E C N S P Y I V G F Y
G A F Y S D G E I S I C M E H M D G G S L D Q V L K K A G R I P E Q I L G K V S I A V I K G L T Y L R E K H K I M H R D V K P S N I L V N S R G E I K L C D F G V S G Q L I D S M A N S F V G T R S Y M S P E R L Q G T H Y S V Q S D I W S M G L S L V E M A V G R
Y P I P P P D A K E L E L M F G C Q V E G D A A E T P P R P R T P G R P L S S Y G M D S R P P M A I F E L L D Y I V N E P P P K I P S G V F S L E F Q D F V N K C L I K N P A E R A D L K Q L M V H A F I K R S D A E E V D F A G W L C S T I G L N Q P S T P T H A A G V
MONO MW: 43411, pl: 6.18
Database = /dbase/IPI/ipi.HUMAN.v3.66_plus_contaminants.fasta
Links: Google Google Scholar NCBI ExPASy SGD
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Representative peptide: **ISELGAGNGGVVFK** (position: 71-84 mass: 1346.7194)

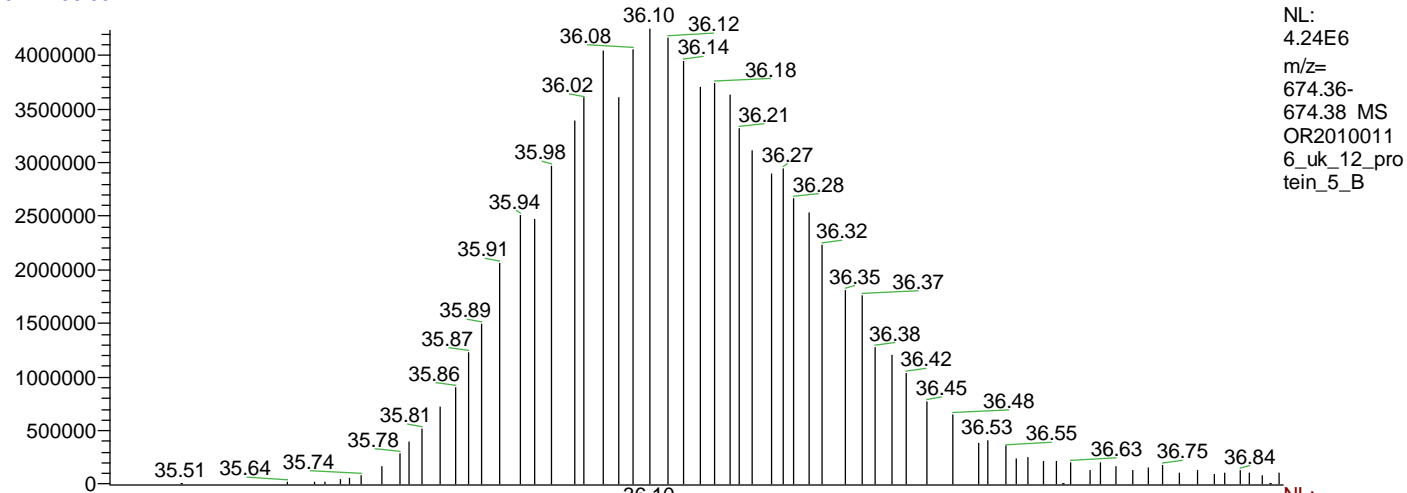
Predicted m/z for +2 ion (M+2H)²⁺ :

Light: 674.3670

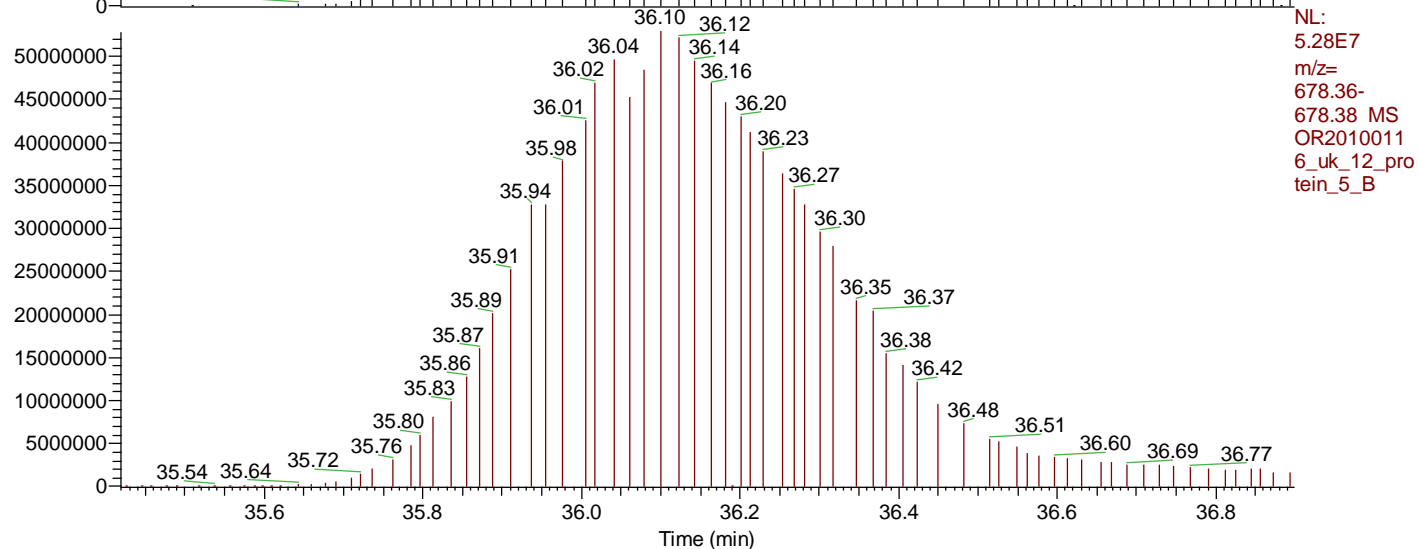
Heavy: 678.3741

HPLC spectra for co-eluted light and heavy peptides of tryptic digested MAP2K1

RT: 35.42 - 36.90



Light

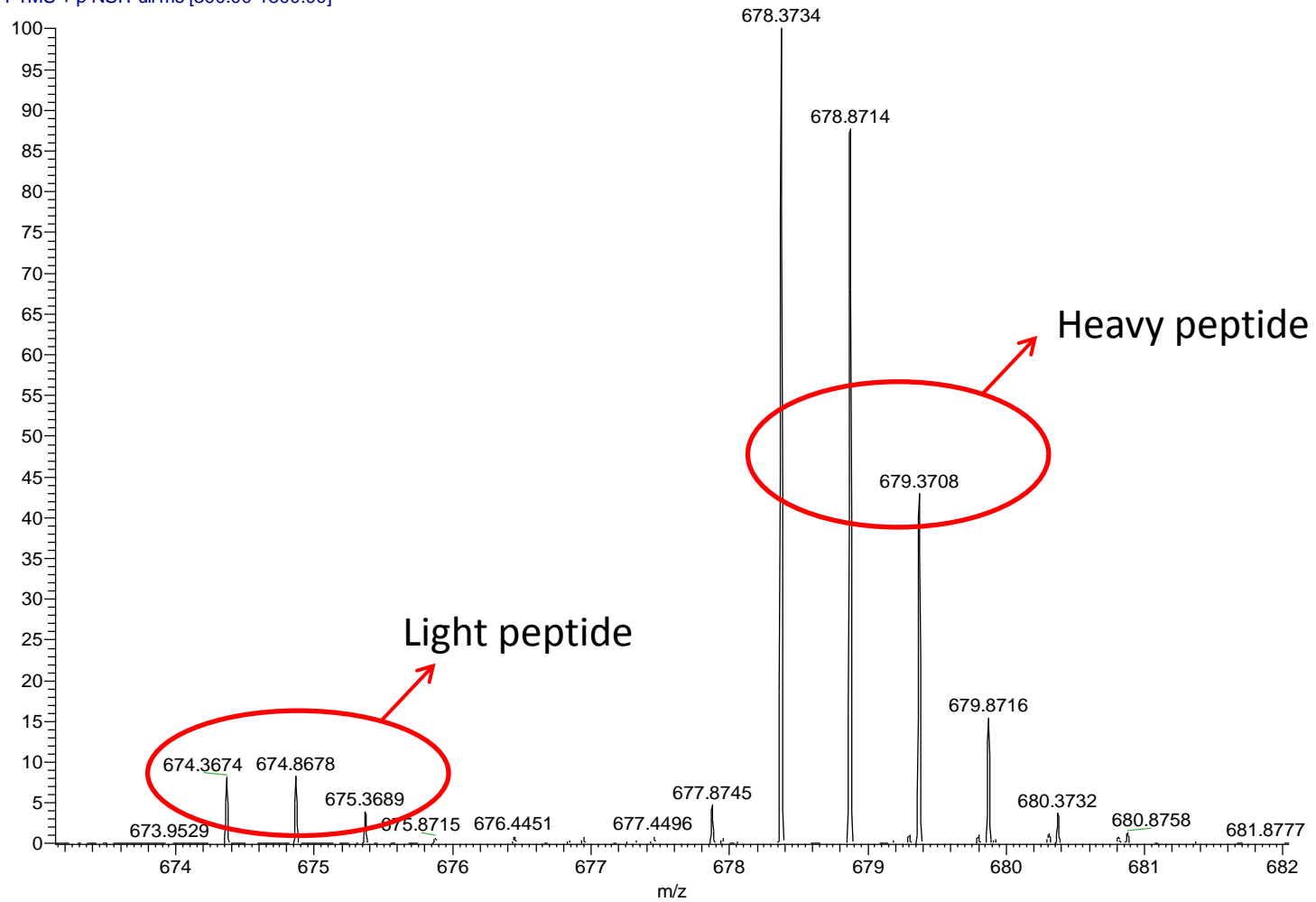


Heavy

Calculated ratio (L/H) by the area under curve (AUC): 0.08

MS spectra of representative light and heavy peptides of tryptic digested MAP2K1

OR20100116_uk_12_protein_5_B #3350 RT: 36.10 AV: 1 NL: 1
T: FTMS + p NSI Full ms [300.00-1800.00]



Peptides identified from MAPK1 MS analysis

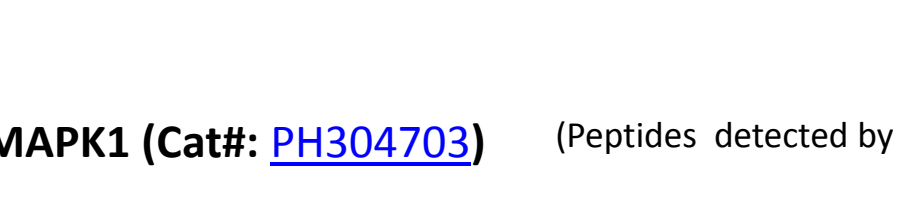
Protein: MAPK1 (Cat#: [PH304703](#)) (Peptides detected by mass spec are highlighted in yellow)

Protein: [IPI00003479](#)

>[IPI00003479](#) IPI:[IPI00003479](#).3|SWISS-PROT:[P28482](#) |TRMBL:[B4DHNO](#);[Q1HBJ4](#);[Q499G7](#) |ENSEMBL:[ENSP00000215832](#) ;[ENSP00000381803](#) |REFSEQ:[NP_002736](#) ;[NP_620407](#)
|H-INV:[HIT000300855](#)|VEGA:[OTTHUMP00000028754](#);[OTTHUMP00000174492](#) Tax Id=9606 Gene Symbol=MAPK1 Mitogen-activated protein kinase 1

MAAAAAGAG PEMV**GQVFD VGPR** TNLST IEGEGAYGMC SAYDNVNR VRAIKKISPFHQTYCQRTLR EIKILLRFRH ENIGINDII RAPTIEQMKD VYIVQDLMET DLYKLLKIQH LSNHICCYFL
YQILRGLKYYI HSAANVLRDL KPSNLLNNT CDLKICDFGL ARVADPDHDH TGFLTEYVAT RNYRQPEIML NSKGYTKSID IWSVGCILAE ML**SNRPFPK** KHYLDQLNHI LGIL**GSPSQE DLNCIINLKA**
RNYLLSLPHK NKVPWNR**LFP** NADSKLDLL DRMLTENPHK RIEVEQALAH PYLEQYDPS DEPIAEAPFK FDMELDDLK EKLKELIFEE TAR**FQPGYRS**

MONO MW: 41363, pl: 6.50
Database = /dbase/IPI/ipi.HUMAN.v3.66_plus_contaminants.fasta
Links: [Google](#) [Google Scholar](#) [NCBI](#) [ExPASy](#) [SGD](#)



Representative peptide: **GQVFDVGPR** (position: 16-24 mass: 973.4981)

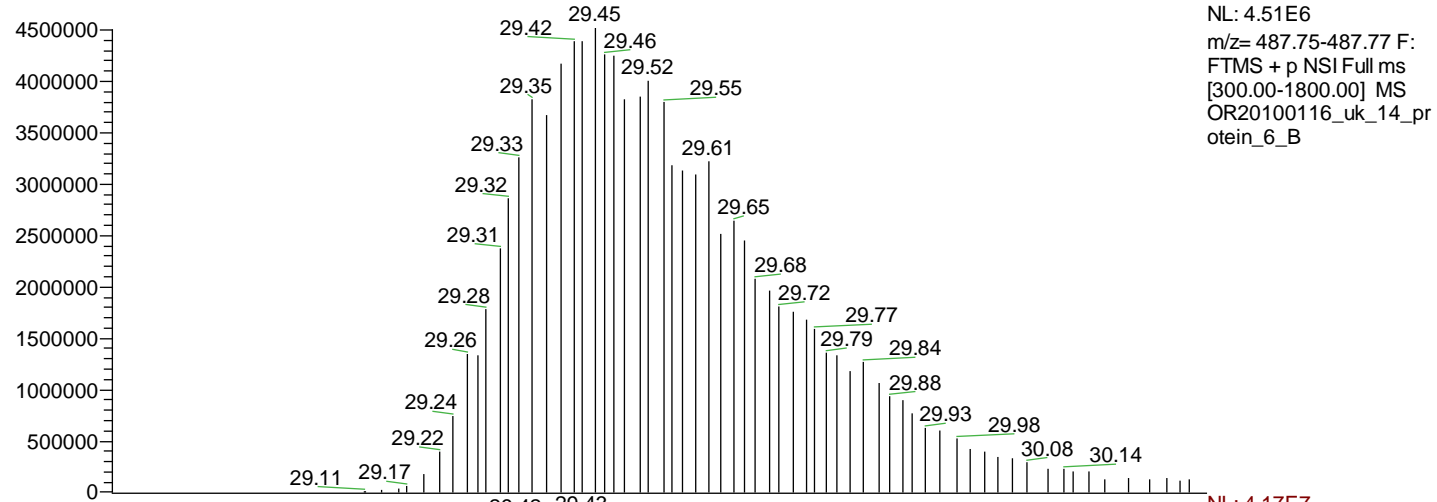
Predicted m/z for +2 ion (M+2H)²⁺ :

Light: 487.7563

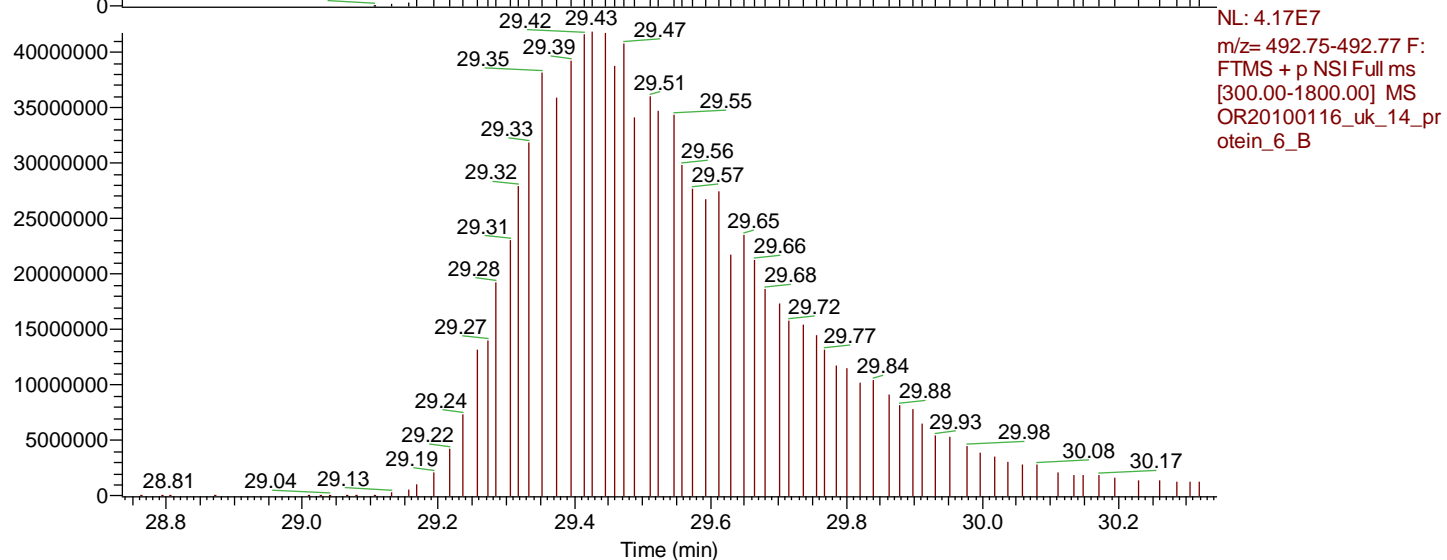
Heavy: 492.7605

HPLC spectra for co-eluted light and heavy peptides of tryptic digested MAPK1

RT: 28.73 - 30.35



Light

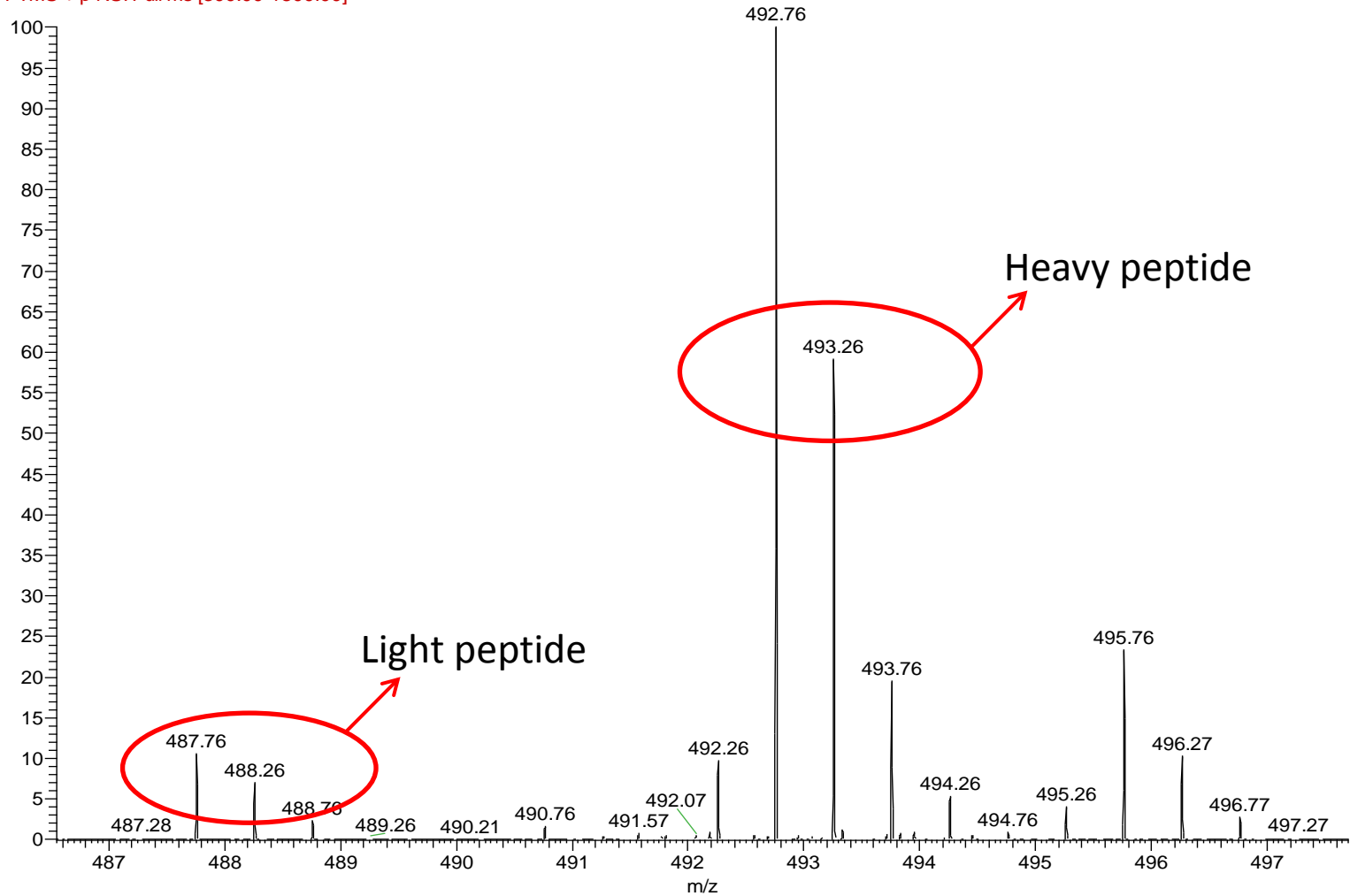


Heavy

Calculated ratio (L/H) by the area under curve (AUC): 0.11

MS spectra of representative light and heavy peptides of tryptic digested MAPK1

OR20100116_uk_14_protein_6_B #2428 RT: 29.43 AV: 1 NL: <
F: FTMS + p NSI Full ms [300.00-1800.00]



Summary

- OriGene C13 and N15 labeled MS protein standards exhibit high purity.
- High percentage of peptide coverage was observed by MS analysis.
- Both the HPLC and MS spectra demonstrated that the overall incorporation efficiency of heavy labeled isotopic amino acids for OriGene heavy labeled Mass Spec protein standards is over 90%.