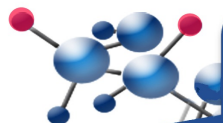


Incorporation efficiency of heavy isotopic amino acids into OriGene's Mass Spec Standards

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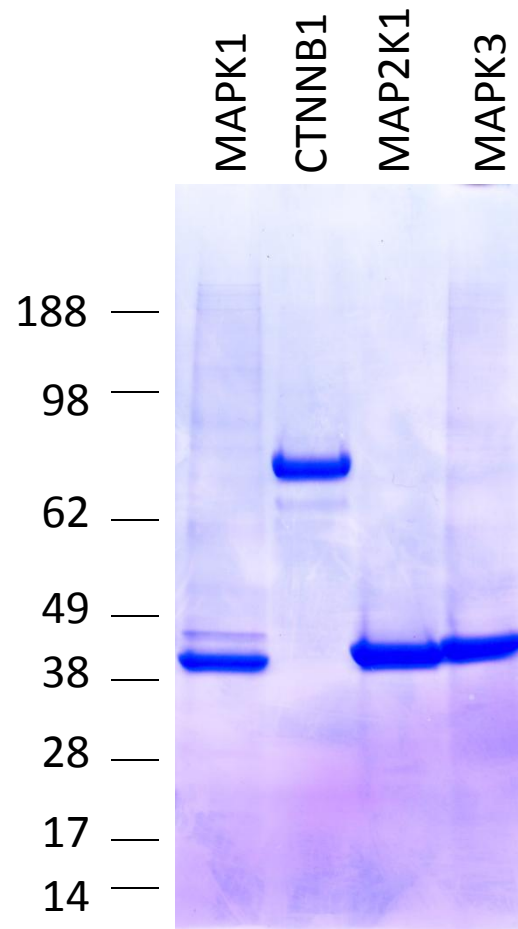
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Introduction

- 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



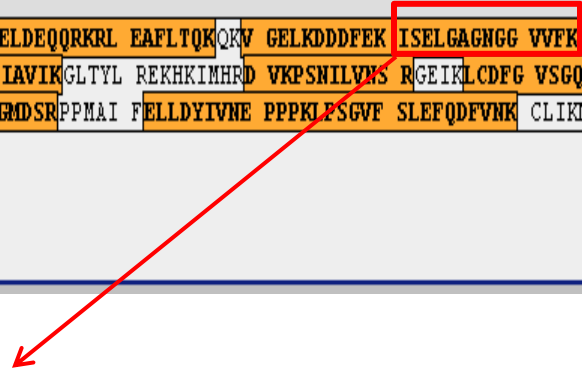
MAP2K1 and MAPK1 were chosen for Mass Spec analysis to determine heavy amino acid incorporation efficiency.

Peptides identified from MAP2K1 MS analysis

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

Protein: IPI00219604

```
>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
```



Representative peptide: **ISELGAGNGGVVFK** (position: 71-84 mass: 1346.7194)

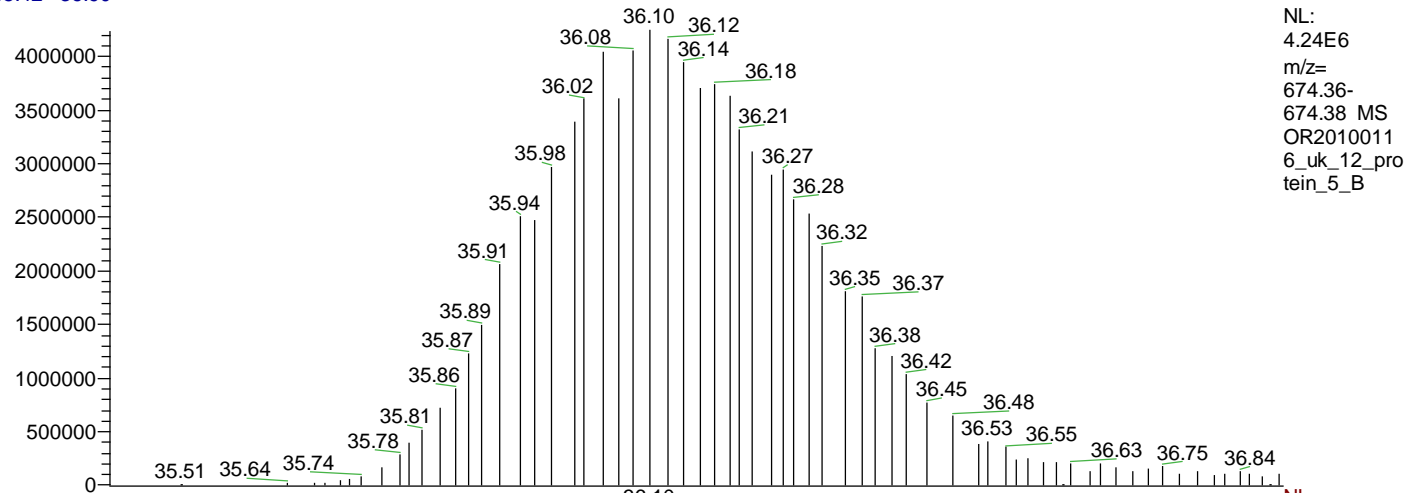
Predicted m/z for +2 ion (M+2H)²⁺ :

Light: 674.3670

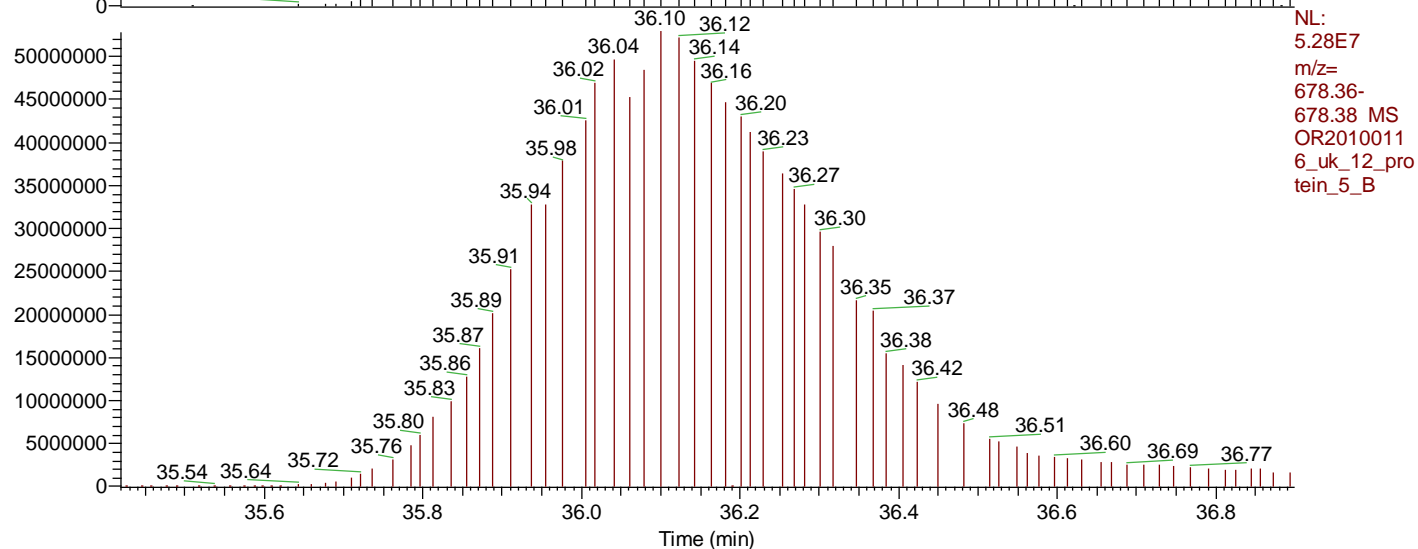
Heavy: 678.3741

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

RT: 35.42 - 36.90



Light

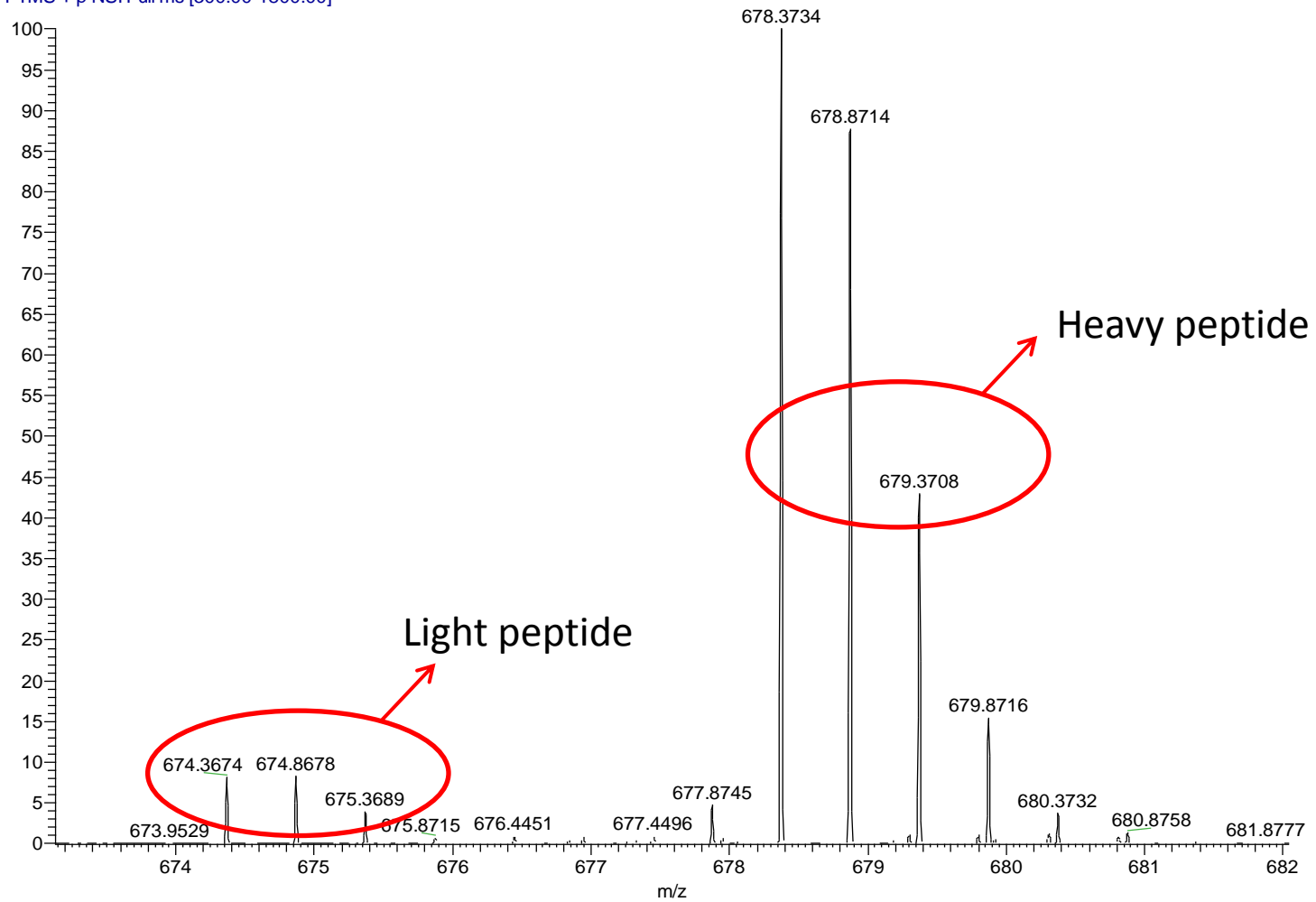


Heavy

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]



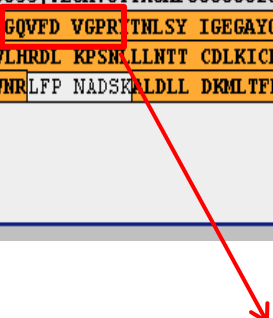
Peptides identified from MAPK1 MS analysis

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

Protein: [IPI00003479](#)

```
>IPI00003479 IPI: IPI00003479.3 | SWISS-PROT: P28482 | TREMBL: 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 PEMVIGQVFD VGPR TNLSTIGEGAYGMC SAYDNVNR VAIK KISPFHQTYCQRTLR EIKILLRFRH ENIGINDII RAPTIEQMKD VYIVQDLMET DLYKLLK TQH LSNHICYFL  
YQILRGLKYYI HSNVLRDL KPSNLLNNT CDLKICDFGL ARVADPDHDH TGFLTEYVAT RNYR APEIML NSKGYTKSID IWSVGCILAE ML SNRPFPK KHYLDQLNHI LGILGSPSQE DLNCIINLKA  
RNYLLSLPHK NKVPWRLFP NADSKLDLL DRMLTENPHK RIEVEQALAH PYLEQYDPS DEPIAEAPFK FDMELDDLK EKLKELIFEE TARFQPGYRS
```

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)

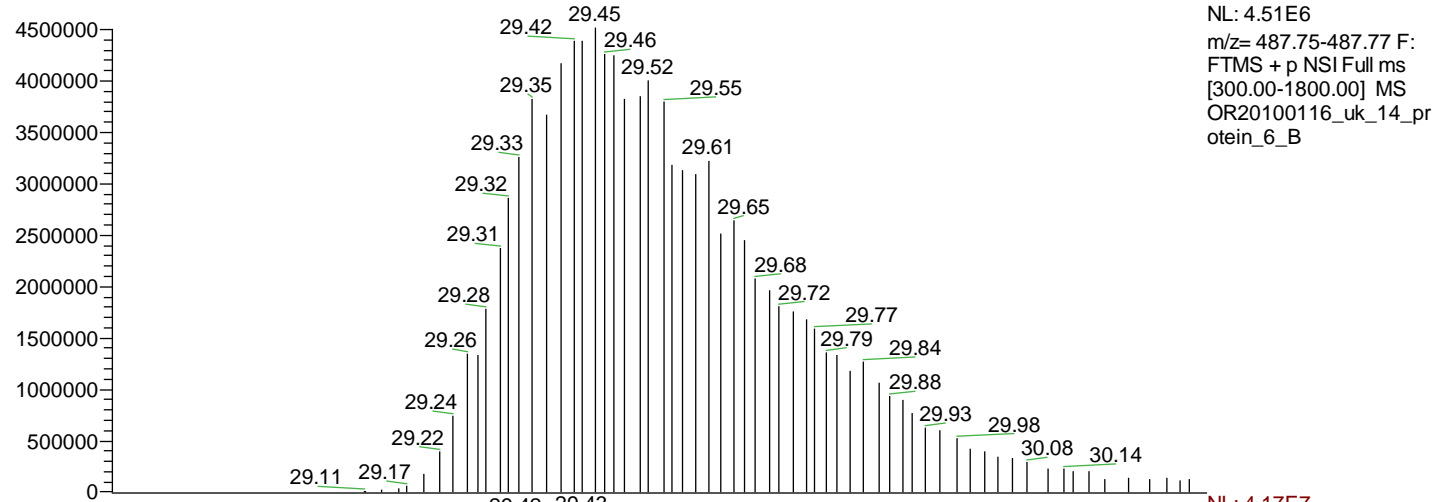
Predicted m/z for +2 ion (M+2H)²⁺ :

Light: 487.7563

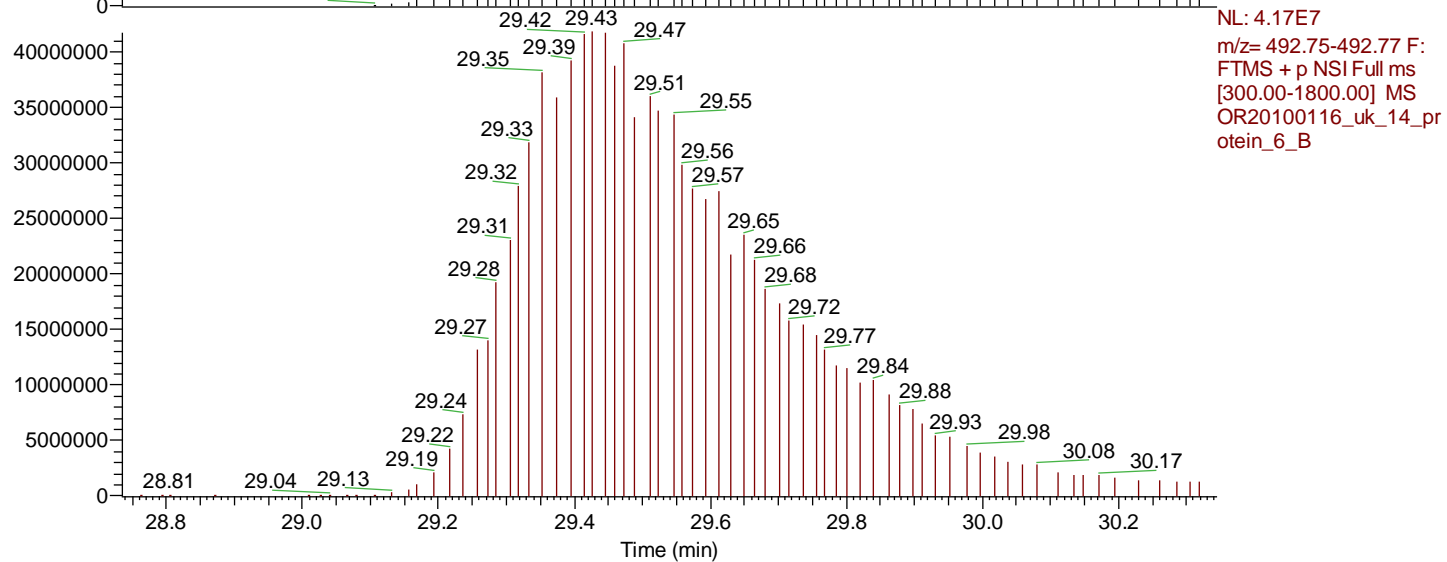
Heavy: 492.7605

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

RT: 28.73 - 30.35



Light

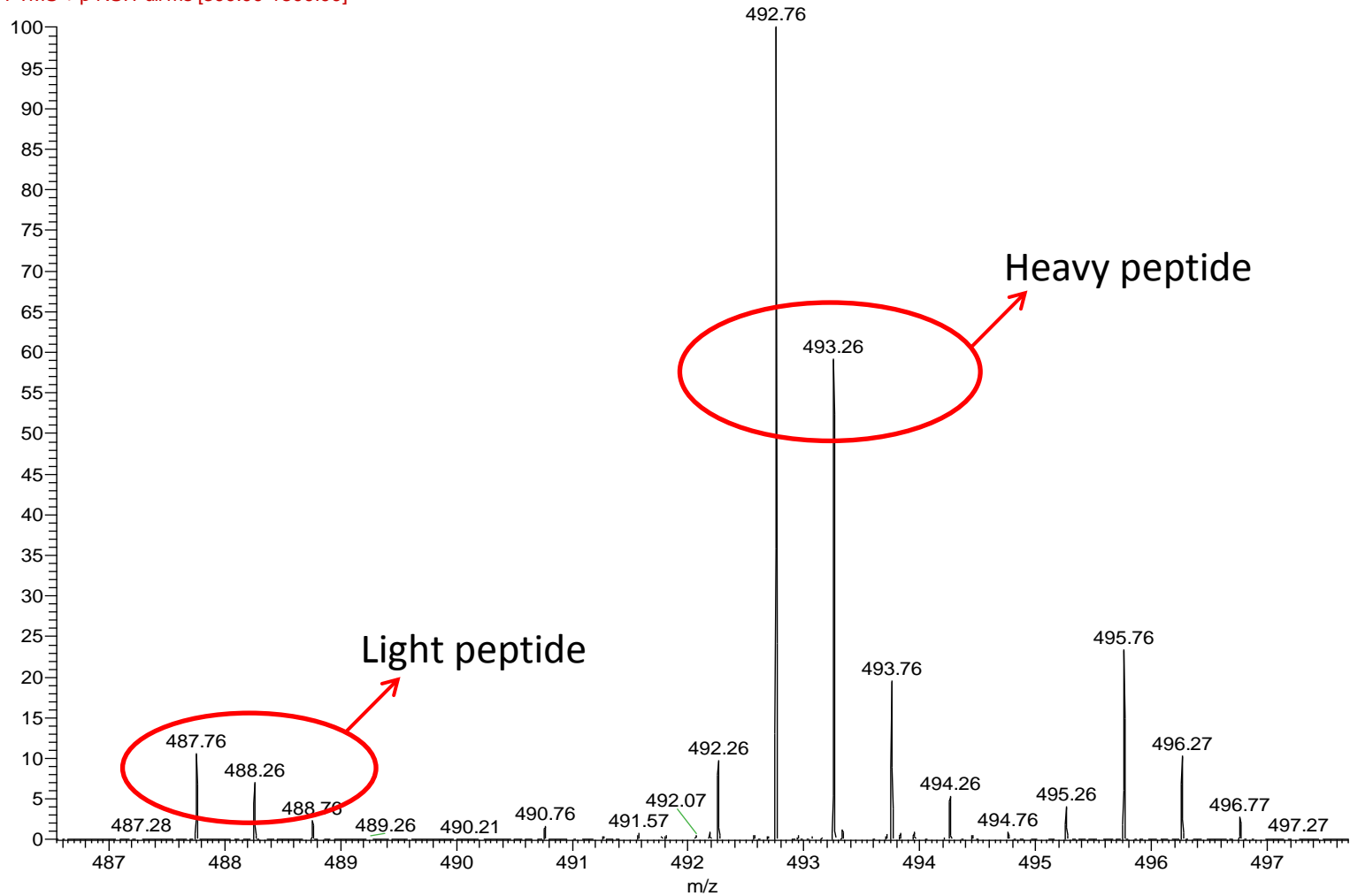


Heavy

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

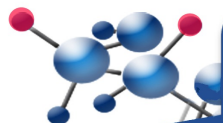
MS spectra of representative light and heavy peptides of tryptic digested MAPK1

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%.



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