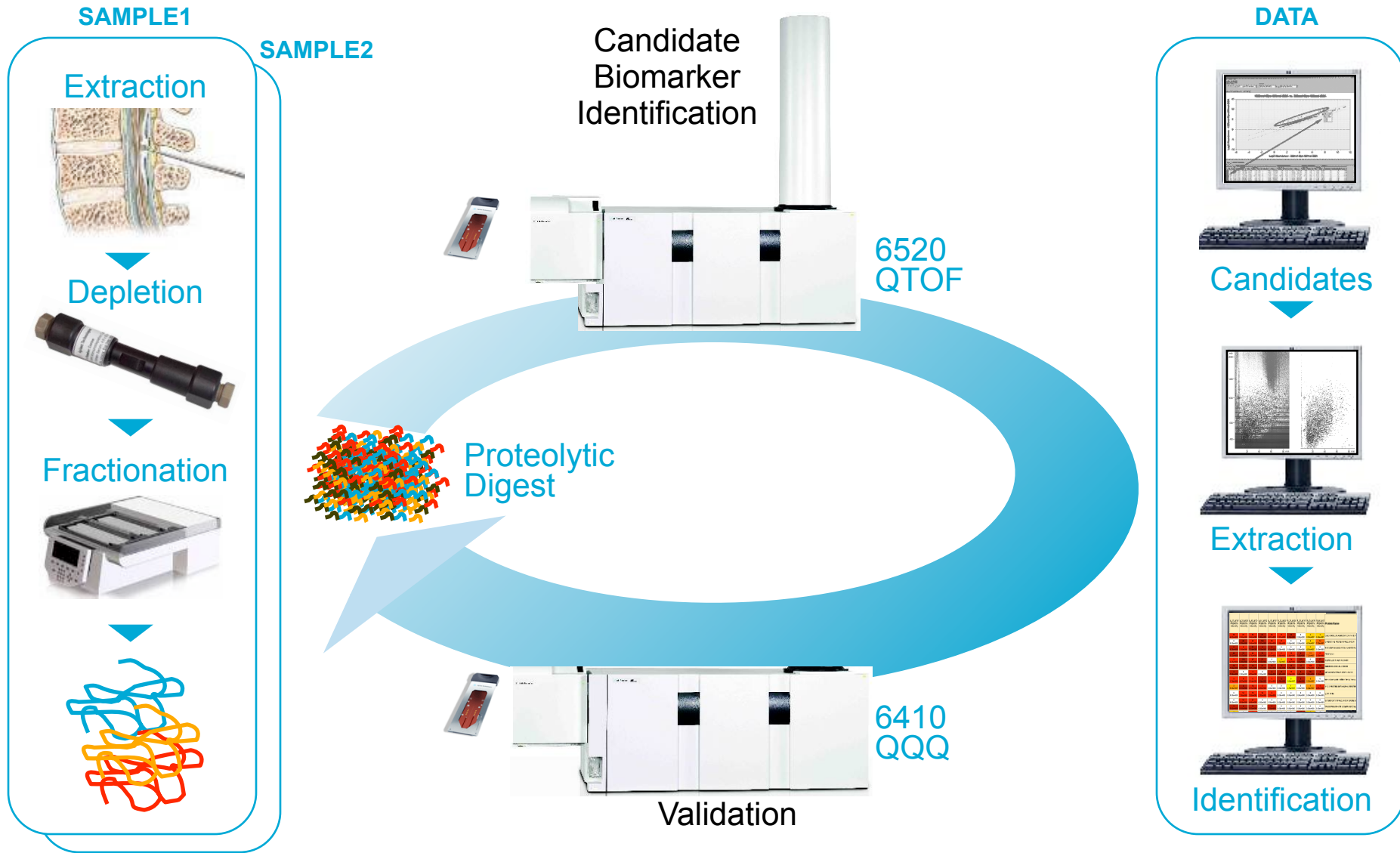


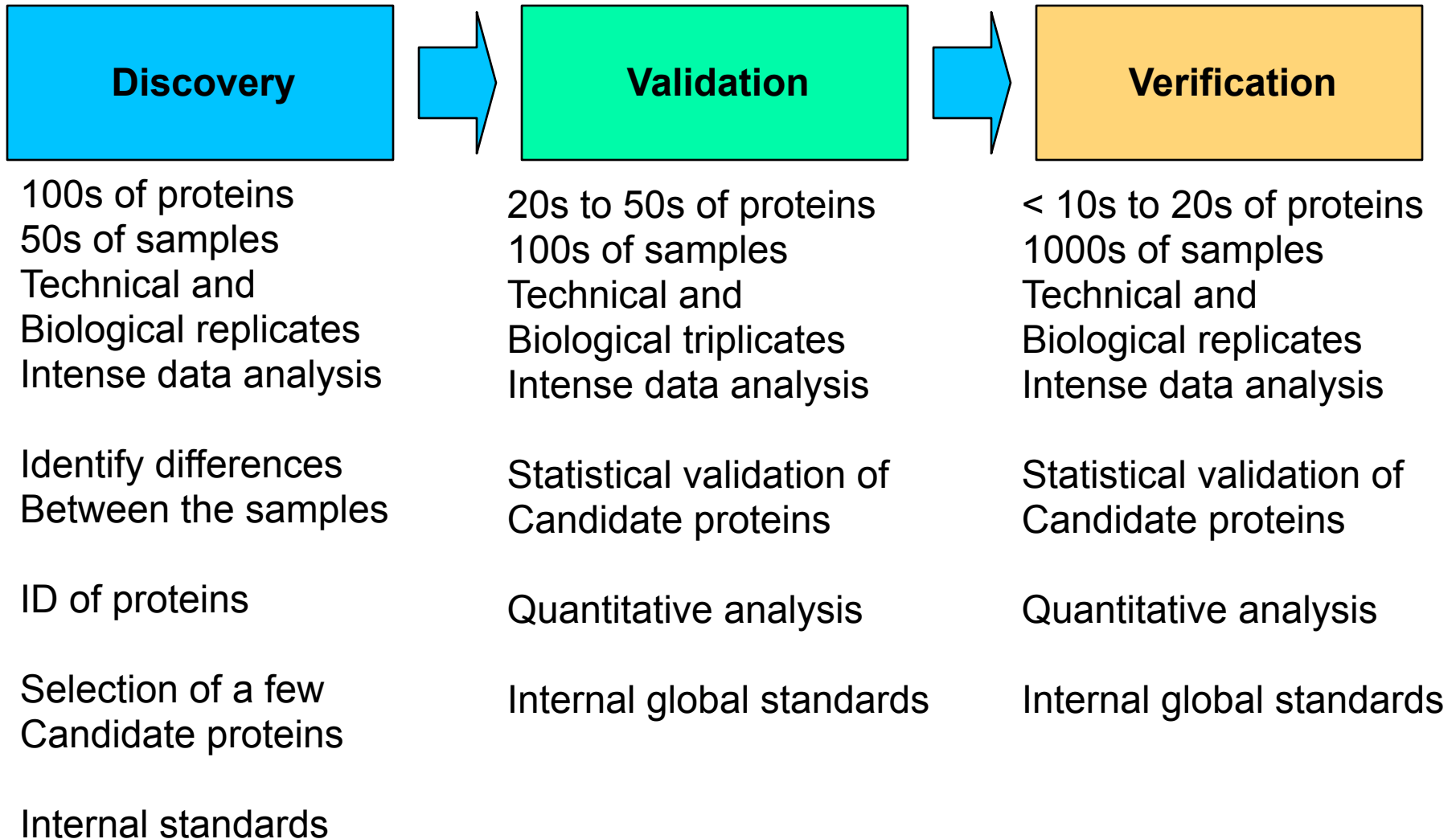
# Peptide Quantitation With An Agilent 6410 QQQ System



# Agilent Proteomics Biomarker Workflow

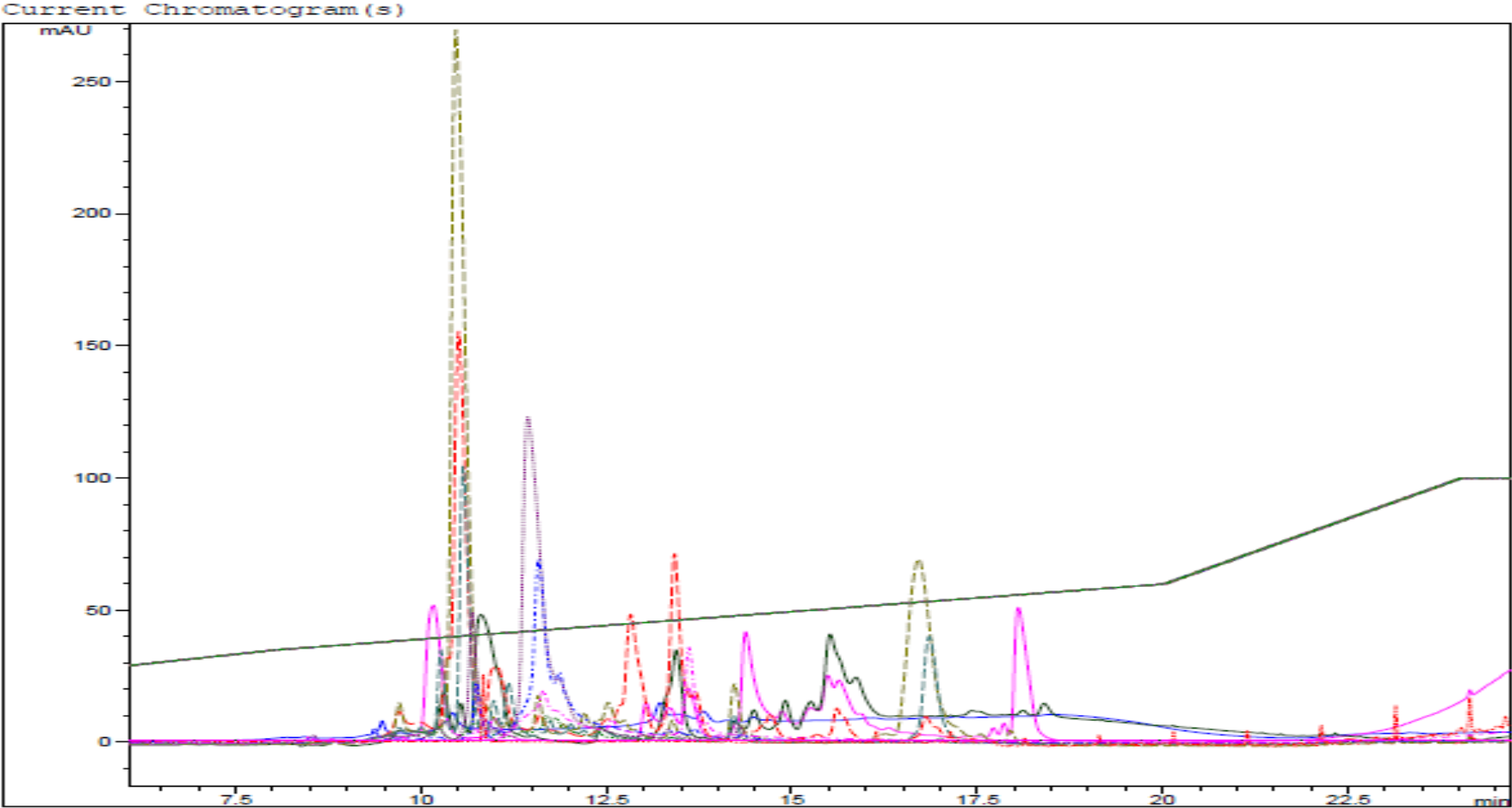


# Discovery, Validation, Verification - Biomarker



# Off-gel and mRP

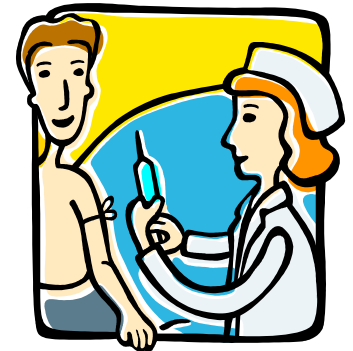
## Fraction from off-gel IEF run on mRP



# Biomarker Validation – Jump From Research Lab to Clinical Lab

	Research	Clinical
<b># of samples</b>	<100	Hundreds - thousands
<b># of proteins</b>	50-500	1-20
<b>Time</b>	Months - years	minutes - hours
<b>Cost</b>	\$100k-1M	\$10-100
<b>CV</b>	20-50%	3-5%

- Reduce cost
- Improve CV
- Reduce the time needed for testing
- Increase throughput



# New HPLC-Chip/MS/MS Technology

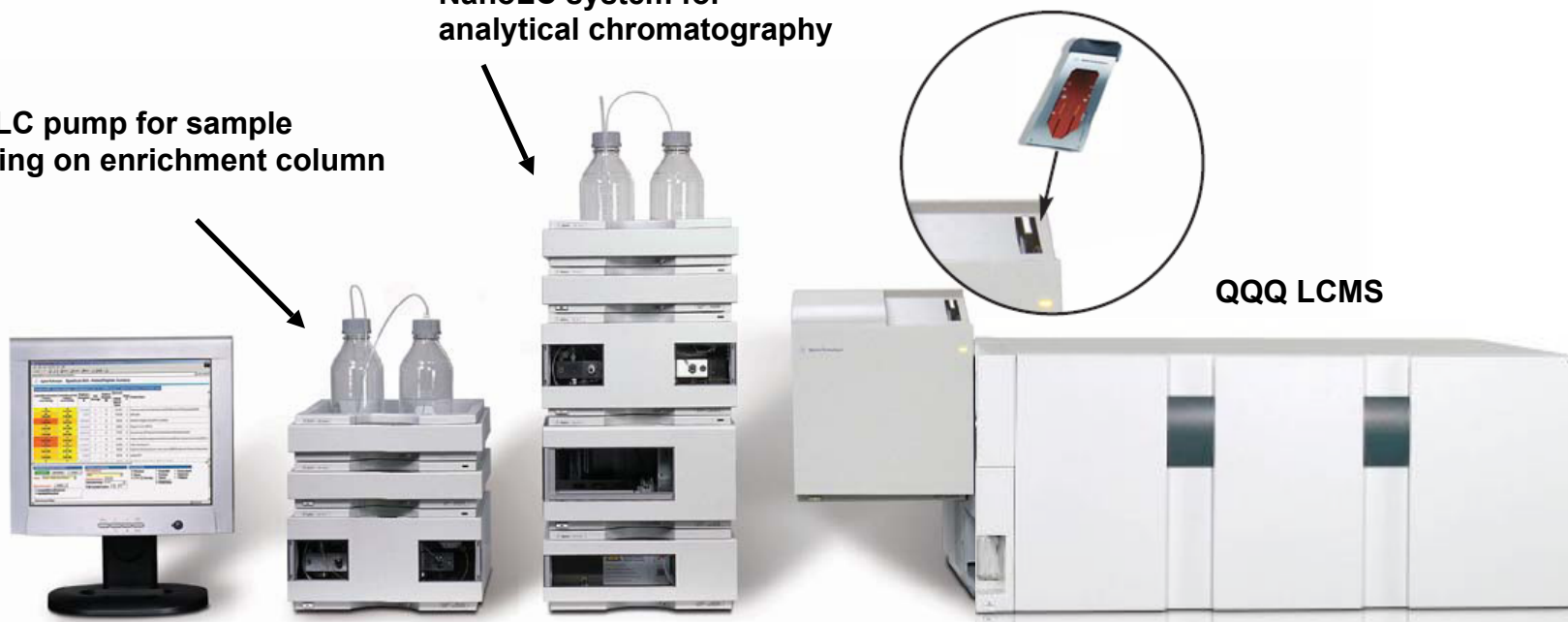
Nanospray chip configuration brings new era in high sensitivity quantitation

CapLC pump for sample loading on enrichment column

NanoLC system for analytical chromatography

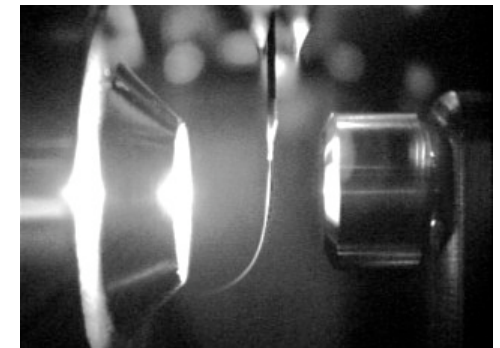
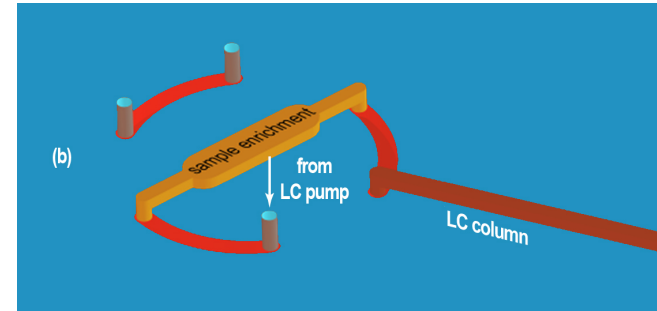
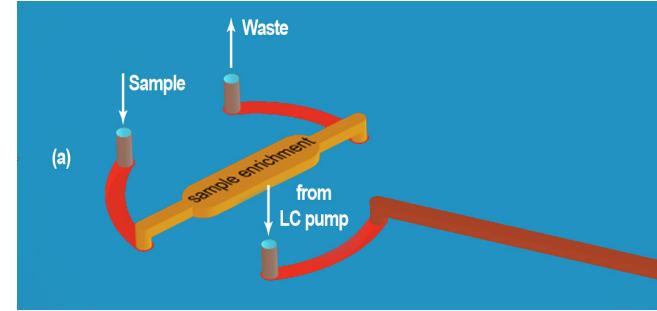
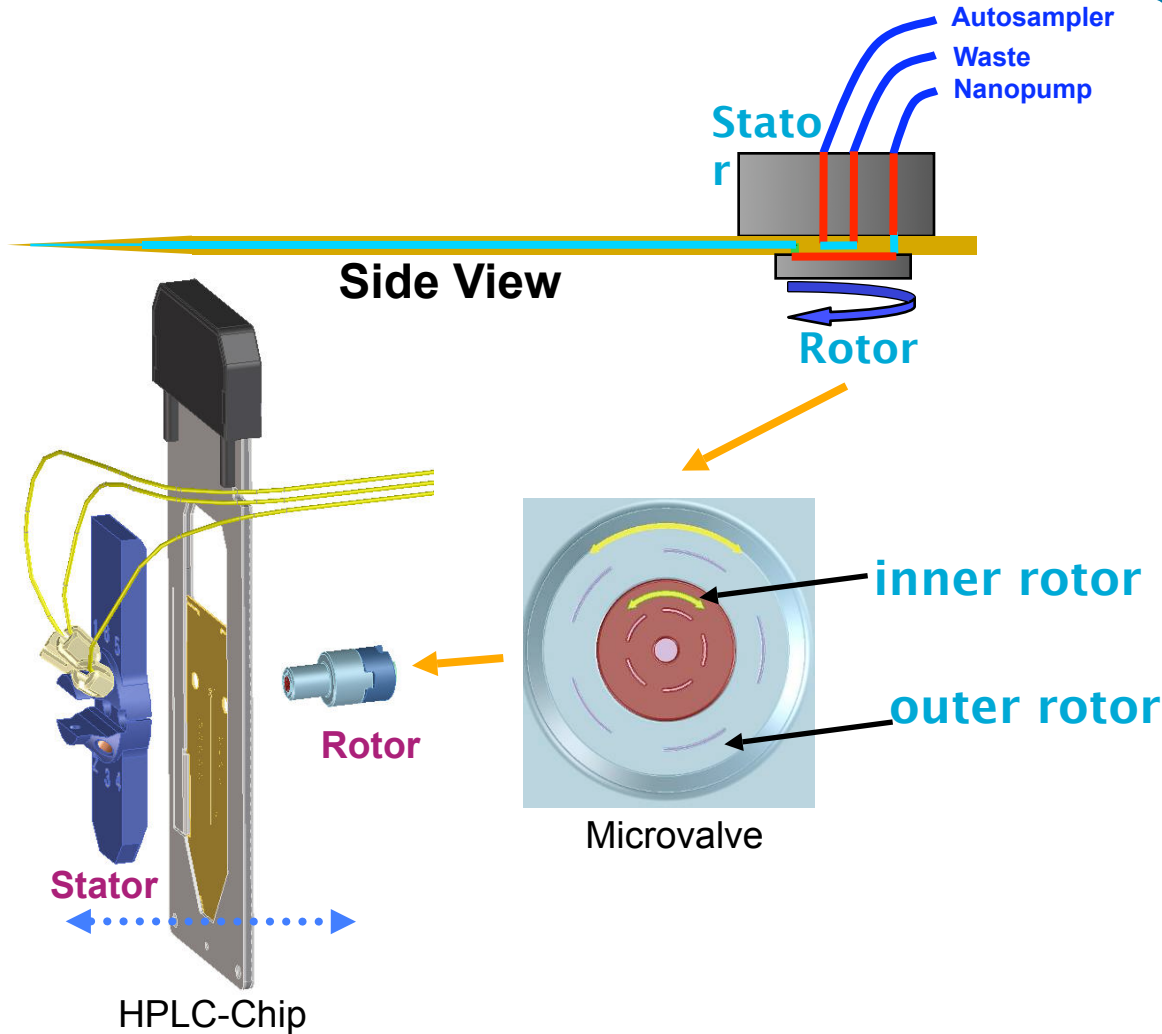
HPLC Chip Cube system

QQQ LCMS

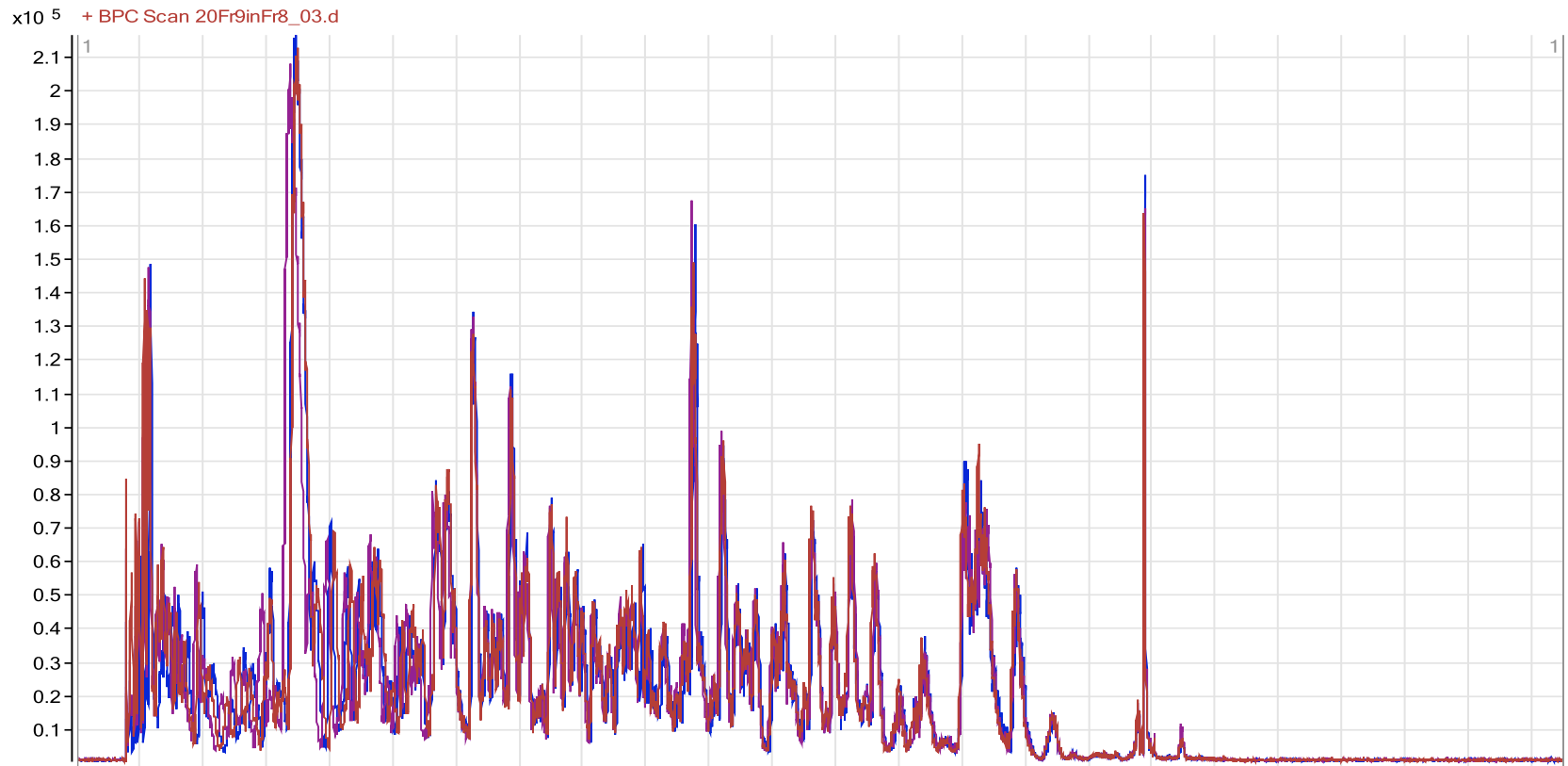


Sensitivity: low -mid amol  
Dynamic range:  $10^3$  -  $10^5$

# HPLC-Chip/MS Interface: Fluid Connections to the HPLC-Chip

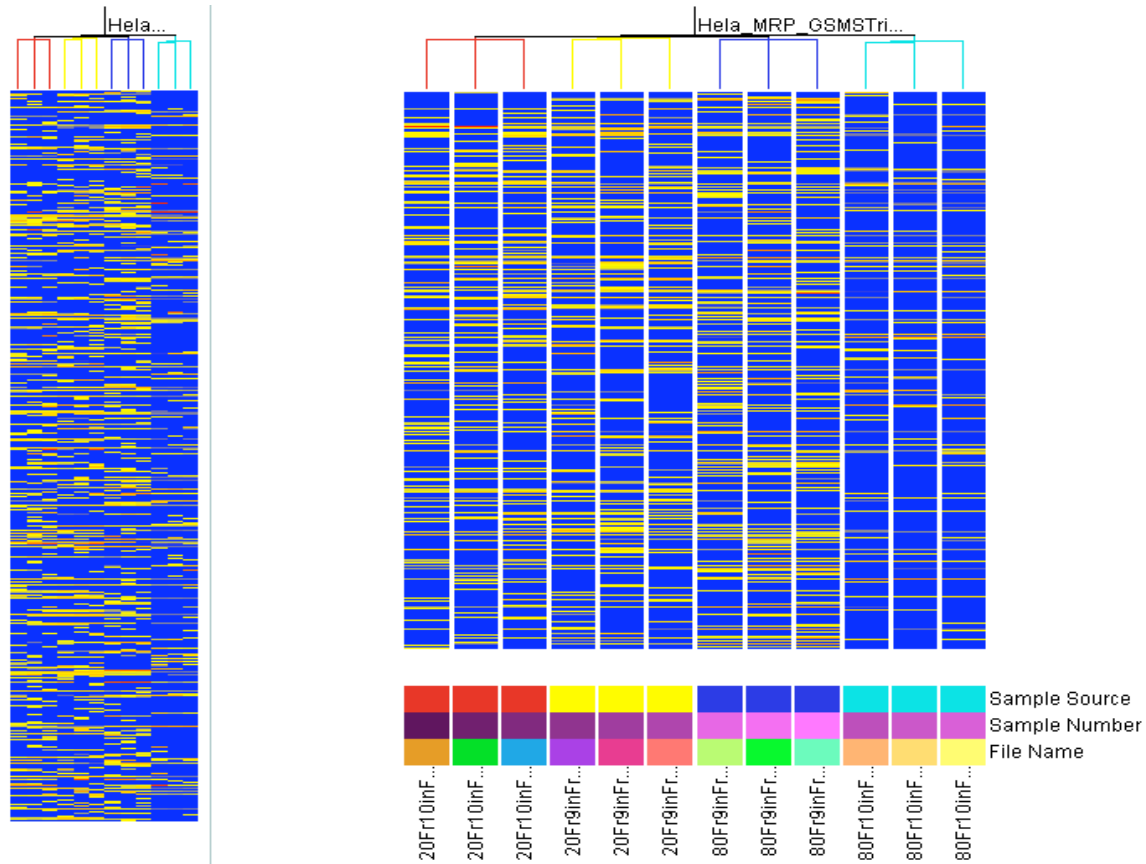


# LC-MS Reproducibility



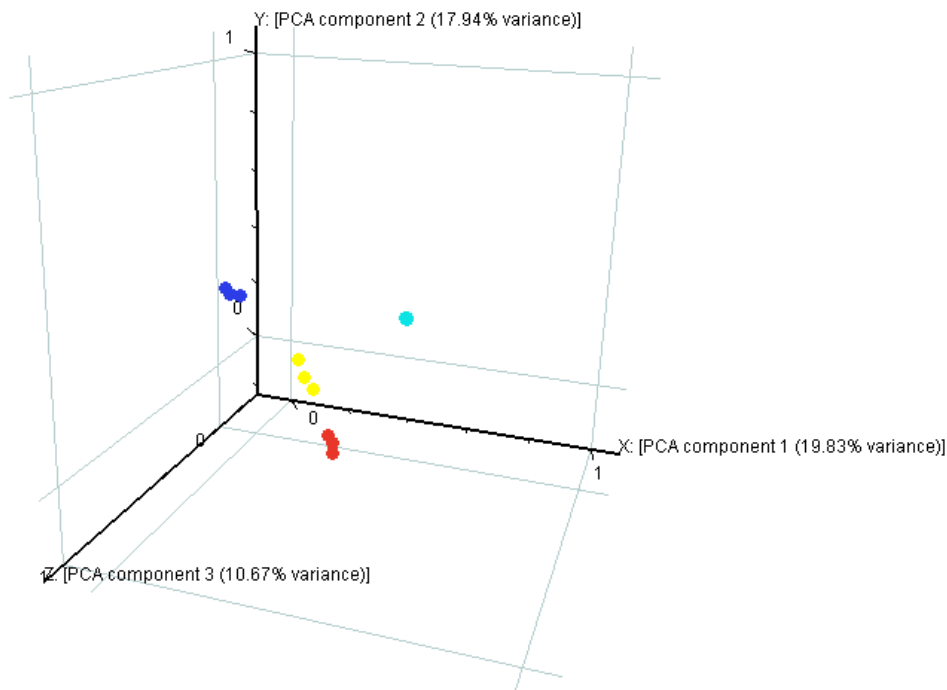
HeLa cell lysate, mRP-subfraction, tryptic digest, run on 15 cm C18 LC-chip (AGILENT) and analysed by MS only on a QTOF (AGILENT 6520)

# Clustering



Selected Condition Tree: HeLa\_MRP\_GSMSTrial (All Samples)    Colored by: HeLa\_MRP\_GSMSTrial, Default Interpretation  
 Branch color parameter: Sample Source    Mass List: all Masses (21545), 1392.7022 selected

# Principal Component Analysis



X-axis: [PCA component 1 (19.83% variance)]  
Y-axis: [PCA component 2 (17.94% variance)]  
Z-axis: [PCA component 3 (10.67% variance)]

Conditions: HeLa\_MRP\_GSMSTrial, Default Interpretation  
Colored by: Parameter Sample Source

# Triple Quadrupole Mass Spectrometer

## *Extending Outstanding Performance*

### 6400 Series – Triple Quad – NEW Functionality

- ✓ Compliance (CFR 21 Part 11 Support)
- ✓ Chip Cube (Ultimate sensitivity)
- ✓ Extended mass range (2000 amu) Upgrade Kit
- ✓ Additional autotune support (Chip Cube)



# Agilent's New *Axial Acceleration* Collision Cell

Overcomes memory or cross-talk effects!

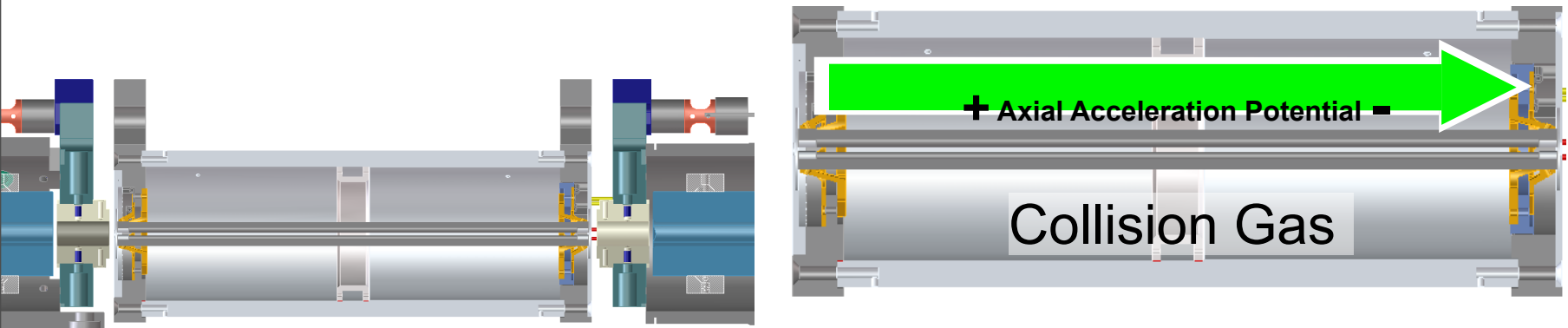
- using high speed ion transport

Maximum sensitivity

- using wide mass range hexapole design

Simple to operate

- no complicated wave forms



# Agilent's New *Axial Acceleration* Collision Cell

Overcomes memory or cross-talk effects!

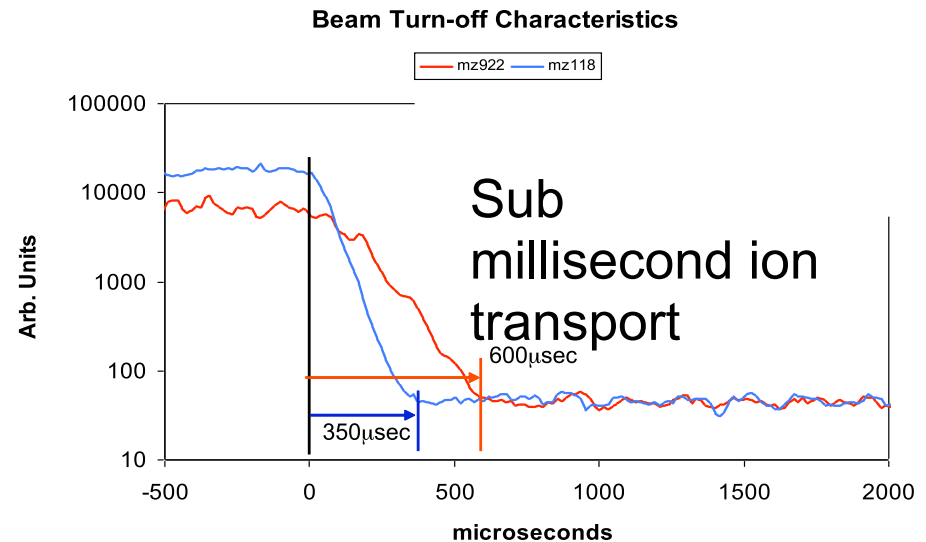
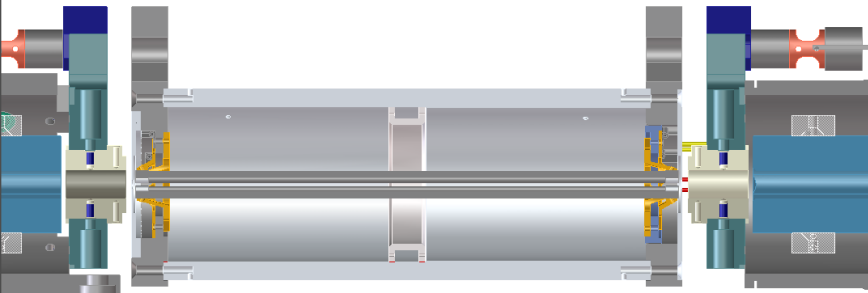
- using high speed ion transport

Maximum sensitivity

- using wide mass range hexapole design

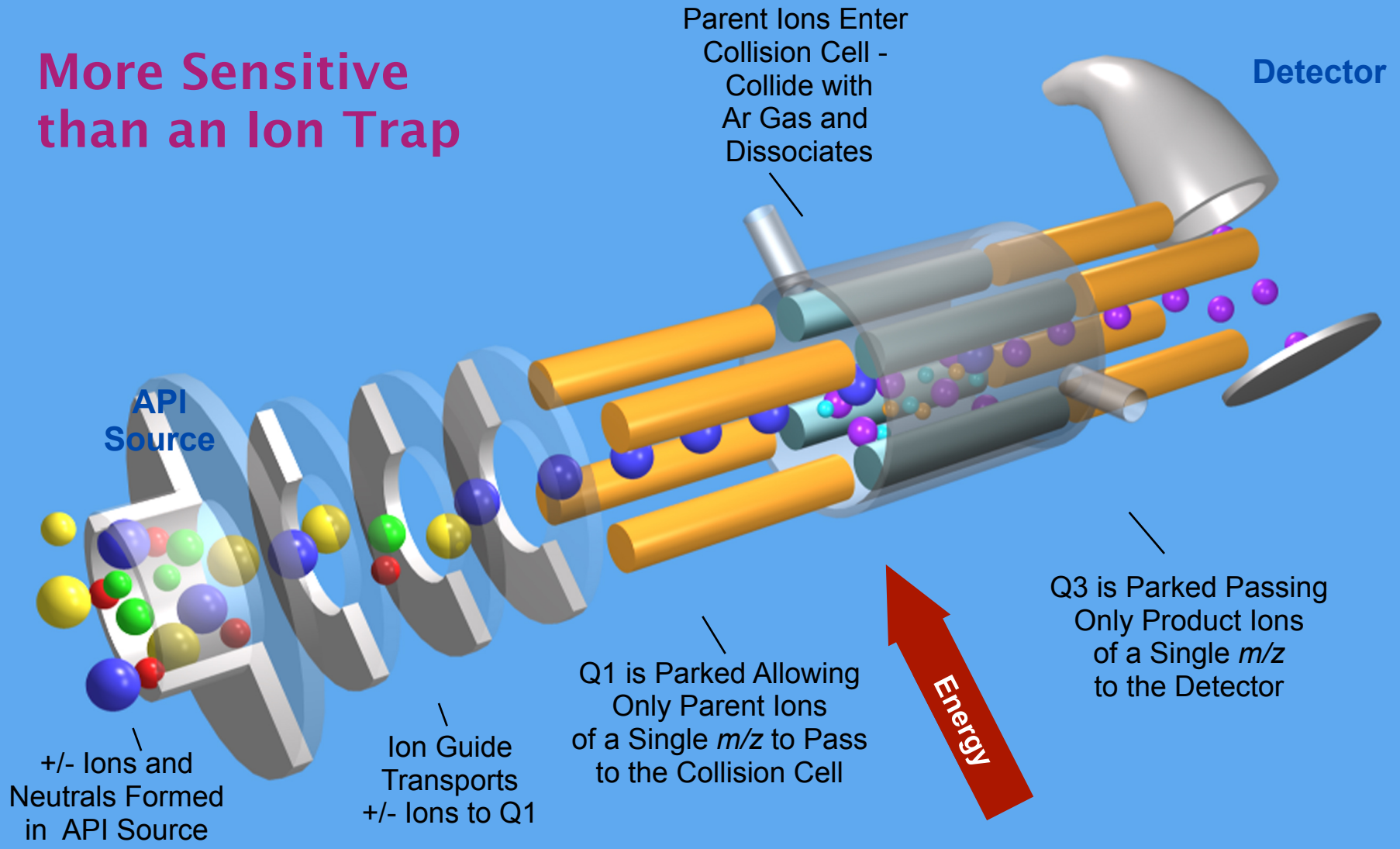
Simple to operate

- no complicated wave forms



# Triple Quadrupole: SRM

**More Sensitive  
than an Ion Trap**



# Mass Hunter Optimizer

## Compound Entry & Method Setup

**Optimizer Editor Panel**

New Project Load Project Save Project SaveAs Project Save Compounds Start Optimization Stop Optimization Pause Optimization

Import From DataBase Import From Excel Export To Excel Hide Instrument Status Panel Hide Plot Control Panel

Compound Setup Precursor Ion Selection Product Ion Selection Optimator Setup

Compounds with known precursor and product ions  Show results (fragmentor and collision energy)

<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Compound Name	Vial Number	Formula	Nominal Mass	Group	Precursor Ion	Fragmentor	Product Ion	Collision Energy	Flagged	CompoundID
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Alprazolam-274, 2 n	P1-A8	C17H13N4Cl	308.0828741543	Pharmaceutical	309.2	135	274.1	35	<input type="checkbox"/>	29276b8c-e2c7-4
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	d5-Alprazolam-210,	P1-A7	C17H8D5N4Cl	313.1142578898	Pharmaceutical	314.2	135	210.1	45	<input type="checkbox"/>	3289d3e3-d4af-4
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Alprazolam-205, 2 n	P1-A8	C17H13N4Cl	308.0828741543	Pharmaceutical	309.2	135	205.1	45	<input type="checkbox"/>	5d49ca38-1293-4
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	d5-Alprazolam-279,	P1-A7	C17H8D5N4Cl	313.1142578898	Pharmaceutical	314.2	135	279.2	35	<input type="checkbox"/>	b767af6d-8bb2-4
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Alprazolam-281, 2 n	P1-A8	C17H13N4Cl	308.0828741543	Pharmaceutical	309.2	135	281.1	30	<input type="checkbox"/>	c5b9eb6f-2aee-4
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	d5-Alprazolam-286,	P1-A7	C17H8D5N4Cl	313.1142578898	Pharmaceutical	314.2	135	286.2	30	<input type="checkbox"/>	fd174aea-658f-40

Compound Setup Precursor Ion Selection Product Ion Selection Optimator Setup

Sample introduction

Manual infusion using syringe

Automatic infusion using Loop injection

Injection (with or without column)

Polarity

Method name

Data file

Fragmentor

Coarse From  To  Step

Fine Step  (This takes more time)

Collision Energy

Coarse From  To  Step

Fine Step  (This takes more time)

# MassHunter MS/MS Method Optimizer

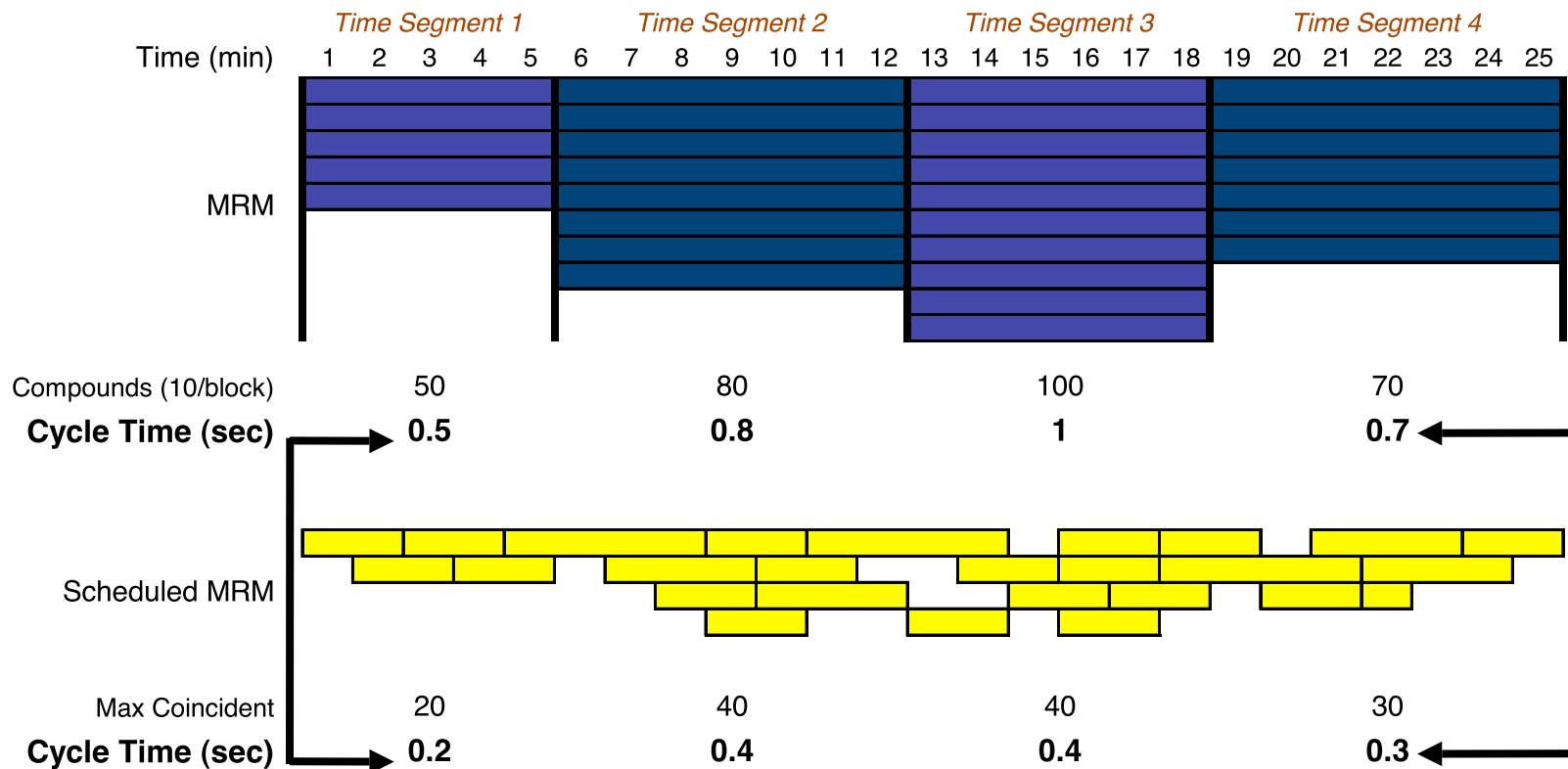
## Basic Steps

- Run 1: The precursor adducts (H<sup>+</sup>, Na<sup>+</sup>, etc) and corresponding Fragmentor value are optimized. Based on user criteria, the optimum precursor mass and Fragmentor value are set.
- Run 2: (optional) fine adjustment of the Fragmentor value.
- Run 3: Coarse product ion scan finds the largest 4 product ions with corresponding Collision Energy. As a default 0, 10, 20, 30, 40 volt CE are surveyed.
- Run 4: Fine adjustment (profile) of each product ion to establish precise mass value.
- Run 5: (optional) fine adjustment of the Collision Energy.

# Scheduled MRM

## Increased Utility and Performance

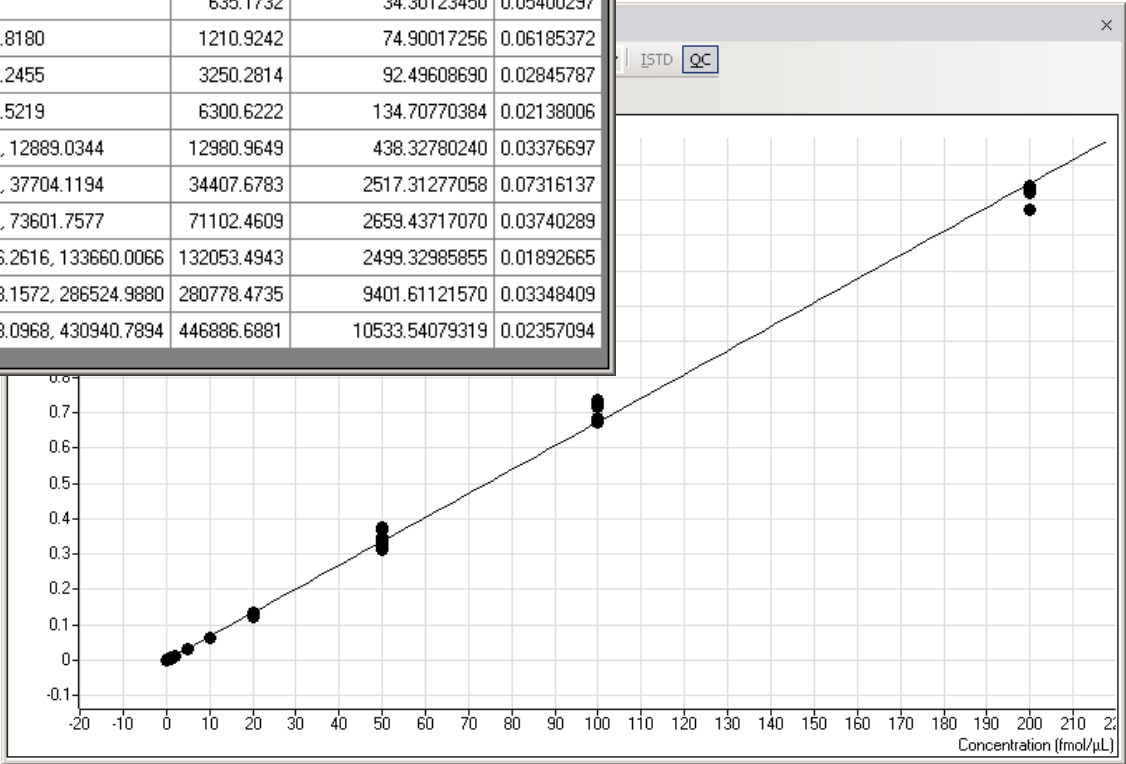
A simple comparison of MRM and Scheduled MRM



2 x shorter cycle times supports narrow chromatographic peaks, more analytes or longer dwell per analyte.

# Reproducibility of MRM response of HSA Peptide from 10 amol to 200 fmol: (n=6)

Level Name	Responses	Average	Standard Deviation	RSD
01	18.9525, 20.8175, 18.3310, 18.0205, 15.5350, 24.2350	19.3153	2.95051515	0.15275573
02	31.0000, 32.6240, 23.9240, 27.0300, 25.1665	27.9489	3.74004406	0.13381722
03	34.2699, 35.7310, 29.5165, 34.1775, 41.3235, 41.9440	36.1604	4.73157795	0.13084969
04	69.2860, 61.8290, 78.9180, 68.6645, 68.6650, 64.6260	68.6648	5.80610139	0.08455723
05	118.6870, 103.1530, 113.0950, 115.2695, 108.4340, 115.5800	112.3698	5.64953978	0.05027634
06	265.0285, 280.5620, 286.1540, 306.0395, 288.6410, 310.7000	289.5208	16.81468530	0.05807764
07	581.9415, 616.4265, 630.1005, 656.1970, 645.0130, 681.3605	635.1732	34.30123450	0.05400297
08	1313.3305, 1251.1835, 1119.7660, 1159.2205, 1161.2268, 1260.8180	1210.9242	74.90017256	0.06185372
09	3339.4015, 3121.2870, 3212.6375, 3288.4455, 3184.6715, 3355.2455	3250.2814	92.49608690	0.02845787
10	6548.3065, 6159.3390, 6304.4050, 6323.6760, 6234.4850, 6233.5219	6300.6222	134.70770384	0.02138006
11	12736.6533, 13629.5363, 13343.9445, 12406.5650, 12880.0559, 12889.0344	12980.9649	438.32780240	0.03376697
12	31088.2466, 32526.2448, 33591.0696, 34795.9507, 36740.4384, 37704.1194	34407.6783	2517.31277058	0.07316137
13	68472.6433, 67181.4113, 71693.2472, 73327.0359, 72338.6699, 73601.7577	71102.4609	2659.43717070	0.03740289
14	127253.4288, 133378.7599, 131800.4635, 133992.0454, 132236.2616, 133660.0066	132053.4943	2499.32985855	0.01892665
15	272565.1274, 283791.4292, 285408.1916, 265992.9477, 290388.1572, 286524.9880	280778.4735	9401.61121570	0.03348409
16	440772.4250, 450591.4181, 443733.2413, 455624.1579, 459658.0968, 430940.7894	446886.6881	10533.54079319	0.02357094



# Limite of quantitation in the low amol range

## Peroxidase spiked into human serum



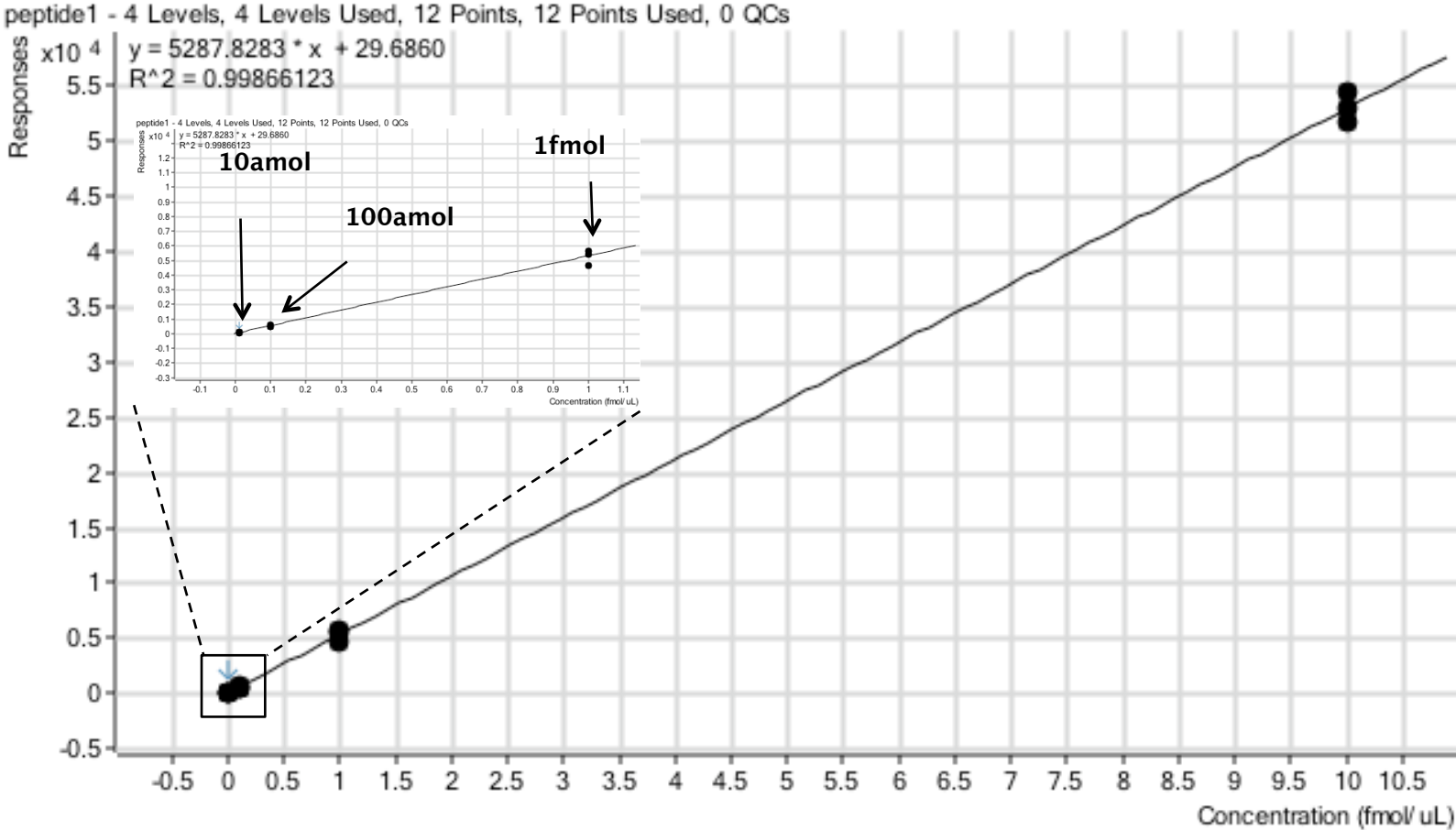
10 amol peroxidase in 1ug serum

100 amol peroxidase in 1ug serum

1 fmol peroxidase in 1ug serum

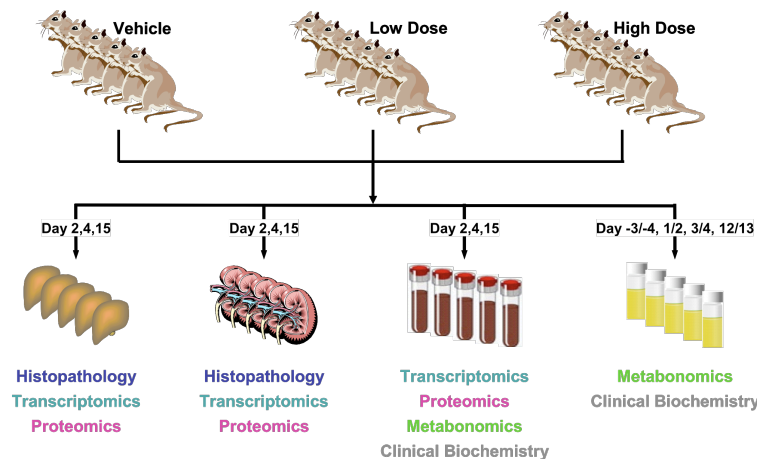
10 fmol peroxidase in 1ug serum

# External Quantitation Curve of Peroxidase Peptide DTIVNELR From 10 amol to 10 fmol Spiked into Human Serum



# Absolute Protein Quantification in the Context of Non-clinical Drug Safety Evaluation

UCD Conway Institute  
University college Dublin  
And  
Agilent Technologies



Collins B. C. et al. ASMS 2008 MPQ 477

# Experimental Design

Catalase was selected based on previous 2D-DIGE data



Peptides and MRM transitions were selected using Peptide Selector in Spectrum Mill and  $^{13}\text{C}$ ,  $^{15}\text{N}$  labeled peptides were synthesized



Rat liver lysate were prepared from rats treated with troglitazone or vehicle control



1 mg of soluble protein extract was reduced, alkylated, acetone precipitated and trypsin digested



The liver digest were spiked with the isotope-labeled peptides and analyzed by Agilent 6410 QQQ system

# Using Spectrum Mill Peptide Selector for Optimising MRM Transitions

The screenshot displays the 'Peptide Selector' web application in a Microsoft Internet Explorer browser window. The browser's address bar shows the URL: `http://smapps/millhtml/mssluiice.htm`. The application interface includes a navigation menu with tabs for 'Spectrum Mill', 'MS Edman', 'Multiple Sequence Aligner', 'Databases', 'MS Digest', and 'Help'. The 'Spectrum Mill' tab is active.

The main interface is divided into several sections:

- Selection:** Contains a green 'Select' button and a checkbox for 'Hide HTML links (better Excel cut/paste)'.
- Digest Parameters:** A dropdown menu is set to 'Trypsin', and 'Maximum # missed cleavages' is set to 0.
- Product Ion Parameters:** A checkbox for 'Show Product Ion Masses' is checked.
- Criteria for Excluding Peptides:**
  - 'Maximum # basic residues (RHK):' is set to 1.
  - 'Minimum peptide MH\*:' is set to 900.0.
  - 'Maximum peptide MH\*:' is set to 2800.0.
  - Peptide exclusion criteria:** Includes checked boxes for 'Has nearby cleavage site within 3 residues', 'Contains peptide N-terminal Gln to pyroGlu', and 'Contains protein N-terminus Acetylated'. Other options are unchecked.
  - AA Composition Filtering:** 'Required AAs:' is set to 'KR' and 'Disallowed AAs:' is set to 'CM'.
- Modifications:** A 'Choose...' button is next to a 'Fixed:' field containing 'Carbamidomethylation (C)'. A 'Variable:' field is empty.
- Protein(s) to Select From:** 'Database:' is set to 'SwissProt'. Below, it says 'Select Peptides from only the Database Entries with the accession numbers below' and 'Allowed delimiters (-,:)' with a list box containing 'P00433'.
- Search Mode:** 'Count Peptide Uniqueness in Database by:' is set to 'None' and 'Species:' is set to 'All'.

The browser's taskbar at the bottom shows 'Local intranet'.

# Using Spectrum Mill Peptide Selector for Optimising MRM Transitions

**Chemically reactive residues**

**(Cys = C, Met = M, Trp = W)**

**Peptides adjacent to multiple cleavage site**

**Chemically unstable residues (Asp-Gly = D-G; Asn-Gly = N-G; N-term Glu = E; N-term Asn = N)**

**Eliminate "LC-incompatible" peptides**

**Uniqueness**

The screenshot shows the 'Peptide Selector' web application in a Microsoft Internet Explorer browser. The interface includes a navigation menu with options like 'Spectrum Mill', 'MS Edman', 'Multiple Sequence Aligner', 'Databases', 'MS Digest', and 'Help'. The main content area is divided into several sections: 'Selection' with a 'Select' button and a 'Hide HTML links' checkbox; 'Digest Parameters' with a 'Digest' dropdown set to 'Trypsin' and a 'Maximum # missed cleavages' field set to '0'; 'Product Ion Parameters' with a 'Show Product Ion Masses' checkbox; 'Criteria for Excluding Peptides' with fields for 'Maximum # basic residues (RHK)' (set to 1), 'Minimum peptide MH\*' (900.0), and 'Maximum peptide MH\*' (2800.0), along with 'Peptide exclusion criteria' checkboxes and 'AA Composition Filtering' options; 'Modifications' with 'Fixed' and 'Variable' modification lists; 'Protein(s) to Select From' with a 'Database' dropdown set to 'SwissProt' and a list of protein accession numbers (e.g., P00433); and 'Search Mode' with a 'Count Peptide Uniqueness in Database by' dropdown set to 'None' and a 'Species' dropdown set to 'All'. The browser's address bar shows the URL 'http://smapps/millhtml/mssluice.htm'.

# Spectrum Mill – Peptide Selector

Agilent Spectrum Mill - Peptide Selector

**Spectrum Mill** | **MS Edman** | **Multiple Sequence Aligner** | **Databases** | **MS Digest** | **Help**

**Selection**

**Select**  Hide HTML links (better Excel cut/paste)

**Digest Parameters** | **Product Ion Parameters**

Digest:  Maximum # missed cleavages:   Show Product Ion Masses

**Criteria for Excluding Peptides**

Maximum # basic residues (RHK):  **Peptide exclusion criteria:** **AA Composition Filtering:**

Minimum peptide MH<sup>+</sup>:   Has nearby cleavage site within  residues Required AAs:

Maximum peptide MH<sup>+</sup>:   Contains peptide N-terminal Gln to pyroGlu Disallowed AAs:

Contains protein N-terminus Acetylated

Contains consensus N-linked glycosylation site

Contains no variable modification

**Modifications**

Choose... Fixed:  Variable:

**Protein(s) to Select From** | **Search Mode**

Database:  Count Peptide Uniqueness in Database by:

Select Peptides from only the Database Entries with the accession numbers below

Allowed delimiters (-,:.)

# Spectrum Mill – Peptide Selector

Agilent Spectrum Mill - Peptide Selector

Spectrum Mill | MS Edman | Multiple Sequence Aligner | Databases | MS Digest | Help

**Select**  Hide HTML links (better Excel cut/paste)

Digest Parameters | Product Ion Parameters

Digest: **Trypsin** Maximum # missed cleavages: **0**  Show Product Ion Masses

Criteria for Excluding Peptides

Maximum # basic residues (RHK): **1** Peptide exclusion criteria:

Minimum peptide MH<sup>+</sup>: **900.0**  Has nearby cleavage site within **3** residues

Maximum peptide MH<sup>+</sup>: **2800.0**  Contains peptide N-terminal Gln to pyroGlu

Contains protein N-terminus Acetylated

Contains consensus N-linked glycosylation site

Contains no variable modification

AA Composition Filtering:

Required AAs: **KR**

Disallowed AAs: **CM**

Modifications

Choose... Fixed: Carbamidomethylation (C) Variable:

Protein(s) to Select From | Search Mode

Database: **SwissProt** Count Peptide Uniqueness in Database by: **Sequence**

Select Peptides from only the Database Entries with the accession numbers below

Allowed delimiters (-,:)

**P04762**

# Spectrum Mill – Peptide Selector

Agilent Spectrum Mill - Peptide Selector

Spectrum Mill | MS Edman | Multiple Sequence Aligner | Databases | MS Digest | Help

**Select**  Hide HTML links (better Excel cut/paste)

**Digest Parameters** | **Product Ion Parameters**

Digest:  Maximum # missed cleavages:   Show Product Ion Masses

**Criteria for Excluding Peptides**

Maximum # basic residues (RHK):  **Peptide exclusion criteria:** **AA Composition Filtering:**

Minimum peptide MH:   Has nearby cleavage site within  residues Required AAs:

Maximum peptide MH:   Contains peptide N-terminal Gln to pyroGlu Disallowed AAs:

Contains protein N-terminus Acetylated

Contains consensus N-linked glycosylation site

Contains no variable modification

**Modifications**

Choose... Fixed: Carbamidomethylation (C) Variable:

**Protein(s) to Select From** | **Search Mode**

Database:  Count Peptide Uniqueness in Database by:

Select Peptides from only the Database Entries with the accession numbers below

Allowed delimiters (-,:)

Species:

# Peptide Selector – Catalase Results

## Peptide Selector Results

Number of database entries: 211104

Database: SwissProt

Exclusion criteria: | Peptide N-terminal Gln to pyroGlu |

Required AA's: KR Disallowed AA's: CM

Exclude if Nearby Cleavage Sites within 3 residues

Digest Used	Max. # Missed Cleavages	Fixed Mods	Peptide N terminus	Peptide C terminus	Masses are	Uniqueness
Trypsin	0	carbamidomethylation	Hydrogen (H)	Free Acid (O H)	Monoisotopic	Count by Sequence

Protein Name: Catalase (EC 1.11.1.6)

Species: RAT

SwissProt Accession #: P04762

MS-Digest Index #: 18675

pI of Protein: 7.15

Protein MW: 59626.3 Da

Amino Acid Composition: A42 C6 D36 E25 F31 G35 H21 I20 K30 L31 M12 N33 P39 Q26 R30 S24 T25 V33 W6 Y21

Protein Name	Acc #	RP-RT	MH+	m/2	# DB peps	Start AA	End AA	Prev. Sequence	Next	b <sub>2</sub>	Y <sub>n</sub> - Y <sub>1</sub>	C-side of Asp, Glu	N-side of Pro
Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK) <a href="#">NLPVEEAGR</a>	(LAQ)	228.14	870.47	757.38	660.33 561.26 432.22 303.18 232.14 175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK) <a href="#">DYPLIPVGG</a>	(LVL)	279.10	886.54	723.48	626.42 513.34 400.26 303.20 204.13 147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR) <a href="#">LAQEDPDYGLR</a>	(DLF)	185.13	1163.53	1092.50	964.44 835.39 720.37 623.31 508.29 345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK) <a href="#">EAETFFPNPFDLTK</a>	(VWP)	201.09	1526.75	1455.72	1326.67 1225.63 1078.56 981.50 834.44 720.3
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK) <a href="#">FYTEDGNWDLVGNNTPIFFIR</a>	(DAM)	311.14	2371.14	2208.07	2107.02 1977.98 1862.95 1805.93 1691.89 1505.8
Catalase (EC 1.11.1.6)	<a href="#">P04762</a>				9								

```

1  ADSRDPASDQ MKQWKEQRAP QKPDVLTIGG GNPIGDGLNI MTAGPRGPLL VQDVVFTIDEM AHFDRERIPE RVVHAKGAGA 80
81  FGYFEVTHDI TRYSKAKVFE HIGKRTPIAV RFSIVAGESG SADIVRDRPG FAVKfyTEDG NWDLVGNNTp IFFIRdAMLF 160
161 PSFIHSQQRN PQTHLKDQDM VWDFWSLQPE SLHQVTFILFS DRGIPDGRHR MNGYGSHTFK LVNANGEAVY CKFHYKTDQG 240
241 IKnLFLVEBAG RLAQEDPDYGLR LRDLFNAIAS GNYPSTFYI QVMIFKEAET FPFNPFDLTK VWPHKDYPLI PVGKLVLNrN 320
321 PANYFAEVEQ MAFDPSNMPP GIEPSPDKML QGRLFAYPDT HRHRLGPNYL QIPVNCPYRA RVANYQRDGP MCMHDNQGGA 400
401 PNYYPNSFSA PEQQSSALEH HSQCASADVkr FNSANEDnvt QVRTIFYTKVL NEEERKRLCE NIANHLKDAQ LFIQRKAVkN 480
481 FTDVHPDYGA RVQALLDQYN SQKPKNAIHT YVQAGSHIAA KGGKANL 526
    
```

The matched peptides cover 12% (64/526 AA's) of the protein.

303.18	232.14	175.12					
303.20	204.13	147.11					
623.31	508.29	345.22	288.20	175.12			
981.50	834.44	720.39	623.34	476.27	361.24	248.16	147.11
1805.93	1691.89	1505.81	1390.78	1277.70	1178.63	1121.61	1007.57

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078



# Peptide Selector – Catalase Results

**Peptide Selector Results**

Number of database entries: 211104  
 Database: **SwissProt**  
 Exclusion criteria: | **Peptide N-terminal Gln to pyroGlu** |  
 Required AA's: **KR** Disallowed AA's: **CM**  
 Exclude if Nearby Cleavage Sites within 3 residues

Digest Used: **Trypsin** Max. # Missed Cleavages: **0** Fixed Mods: **carbamidomethylation** Peptide N terminus: **Hydrogen (H)** Peptide C terminus: **Free Acid (O H)** Masses are **Monoisotopic** Uniqueness Count by **Sequence**

Protein Name: **Catalase (EC 1.11.1.6)**  
 Species: **RAT**  
 SwissProt Accession #: **P04762**  
 MS-Digest Index #: **18675**  
 pI of Protein: **7.15**  
 Protein MW: **59626.3 Da**  
 Amino Acid Composition: **A42 C93 D36 E25 F31 G35 H21 I20 K30 L31 M12 N33 P35 Q26 R30 S24 T25 V33 W6 Y21**

Protein Name	Acc #	RP-RT	MH+	m/2	# DB peps	Start AA	End AA	Prev. Sequence	Next	b <sub>2</sub>	y <sub>n</sub> - y <sub>1</sub> C-side of Asp, Glu N-side of Pro							
Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK) <a href="#">NLPVEEAGR</a>	(LAQ)	228.14	870.47	757.38	660.33	561.26	432.22	303.18	232.14	175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK) <a href="#">DYPLIPVVGK</a>	(LVL)	279.10	886.54	723.48	626.42	513.34	400.26	303.20	204.13	147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR) <a href="#">LAQEDPDYGLR</a>	(DLF)	185.13	1163.53	1092.50	964.44	835.39	720.37	623.31	508.29	345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK) <a href="#">EAETFFPNPFDLTK</a>	(VWP)	201.09	1526.75	1455.72	1326.67	1225.63	1078.56	981.50	834.44	720.3
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK) <a href="#">FYTEDGNWDLVGNNTPIFFIR</a>	(DAM)	311.14	2371.14	2208.07	2107.02	1977.98	1862.95	1805.93	1691.89	1505.8
Catalase (EC 1.11.1.6)	<a href="#">P04762</a>				9													

1	ADSRDPASDQ	MKQWKEQRAP	QKPDVLTIGG	GNPIGDGLNI	MTAGPRGPLL	VQDVVFTIDEM	AHFDRERIPE	RVVHAKGAGA	80
81	FGYFEVTHDI	TRYSKAKVFE	HIGKRTPIAV	RFSIVAGESG	SADIVRDRPRG	FAVKFYTEDG	NWDLVGNNTP	IFFIRDAMLF	160
161	PSFIHSQQRN	PQTHLKDPDFM	VWDFWSLCEP	SLHQVTFILFS	DRGIPDGRHR	MNGYGSHTFK	LVNANGEAVY	CKFHYKTDQG	240
241	IKNLFVEBAG	RLAQEDPDYQ	LRDLFNAIAS	GNYPSTFYI	QVMIFKEAET	FPFNPFDLTK	VWPHKDYPLI	PVGKLVLNRN	320
321	PANYFAEVEQ	MAFDPSNMPP	GIEPSPDKML	QGRLFAYPDT	HRHRLGPNYL	QIPVNCPYRA	RVANYQRDGP	MCMHDNQGGA	400
401	PNYYPNSFSA	PEQQSSALEH	HSQCSADVQR	FNSANEDNVT	QVRTIFYTKVL	NEEERKRLCE	NIANHLKDAQ	LFIQRKAVKN	480
481	PTDVHPDYGA	RVQALLDQYN	SQKPKNAIHT	YVQAGSHIAA	KGKANL				526

The matched peptides cover 12% (64/526 AA's) of the protein.

303.18	232.14	175.12					
303.20	204.13	147.11					
623.31	508.29	345.22	288.20	175.12			
981.50	834.44	720.39	623.34	476.27	361.24	248.16	147.11
1805.93	1691.89	1505.81	1390.78	1277.70	1178.63	1121.61	1007.57

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078

# Peptide Selector – Catalase Results

**Peptide Selector Results**

Number of database entries: 211104  
 Database: **SwissProt**  
 Exclusion criteria: | **Peptide N-terminal Gln to pyroGlu** |  
 Required AA's: **KR** Disallowed AA's: **CM**  
 Exclude if Nearby Cleavage Sites within 3 residues

Digest Used: **Trypsin** Max. # Missed Cleavages: **0** Fixed Mods: **carbamidomethylation** Peptide N terminus: **Hydrogen (H)** Peptide C terminus: **Free Acid (O H)** Masses are **Monoisotopic** Uniqueness Count by **Sequence**

Protein Name: **Catalase (EC 1.11.1.6)**  
 Species: **RAT**  
 SwissProt Accession #: **P04762**  
 MS-Digest Index #: **18675**  
 pI of Protein: **7.15**  
 Protein MW: **59626.3 Da**  
 Amino Acid Composition: **A42 C93 D36 E25 F31 G35 H21 I20 K30 L31 M12 N33 P35 Q26 R30 S24 T25 V33 W6 Y21**

Protein Name	Acc #	RP-RT	MH+	m/2	# DB peps	Start AA	End AA	Prev. Sequence	Next	b <sub>2</sub>	Y <sub>n</sub> - Y <sub>1</sub> C-side of Asp, Glu N-side of Pro
Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK) <a href="#">NLPVEEAGR</a>	(LAQ)	228.14	870.47 <b>757.38</b> 660.33 561.26 <b>432.22</b> <b>303.18</b> 232.14 175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK) <a href="#">DYPLIPVVGK</a>	(LVL)	279.10	<b>886.54</b> <b>723.48</b> 626.42 513.34 <b>400.26</b> 303.20 204.13 147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR) <a href="#">LAQEDPDYGLR</a>	(DLF)	185.13	1163.53 1092.50 964.44 <b>835.39</b> <b>720.37</b> 623.31 <b>508.29</b> 345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK) <a href="#">EAETFPFNFELTK</a>	(VWP)	201.09	<b>1526.75</b> <b>1455.72</b> <b>1328.67</b> <b>1225.63</b> <b>1078.56</b> <b>720.37</b> 981.50 834.44
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK) <a href="#">FYTEDGNVWVGNNTPIFFIR</a>	(DAM)	311.14	2371.14 2208.07 2107.02 <b>1977.98</b> <b>1862.95</b> 1805.93 1691.89 1505.8
Catalase (EC 1.11.1.6)	<a href="#">P04762</a>				9						

1	ADSRDPASDQ	<b>MKQWKEQ</b> RAP	QKPDVLTIGG	GNPIGDKLN	MTAGPRGPLL	VQDVVFTIDEM	AHFDRERIPE	<b>R</b> VVHAKGAGA	80		
81	FGYFEVTHDI	TRYS <b>KAK</b> VFE	HIGKRTPIAV	<b>R</b> FSIVAGESG	SADIVRDRPRG	FAV <b>KFYTEDG</b>	<b>N</b> WDLVGN <b>N</b> TP	<b>I</b> FFIR <b>D</b> AMLF	160		
161	PSFIHSQ <b>KRN</b>	PQTHL <b>KD</b> PFM	VWDFWSL <b>C</b> PE	SLHQVTFILFS	DRGIPDGHRH	MNGYGSHT <b>F</b> K	LVNANGEAVY	<b>C</b> K <b>F</b> HY <b>K</b> TDQ <b>G</b>	240	<b>303.18</b>	232.14 175.12
241	<b>I</b> <b>K</b> <b>N</b> L <b>F</b> VE <b>B</b> AG	<b>R</b> LAQED <b>P</b> DY <b>G</b> L <b>R</b>	<b>L</b> RD <b>L</b> FN <b>A</b> IAS	GNYP <b>S</b> WTFYI	QVM <b>F</b> K <b>E</b> AB <b>T</b>	<b>F</b> PF <b>N</b> PF <b>D</b> L <b>T</b> K	V <b>W</b> PH <b>K</b> DY <b>P</b> L <b>I</b>	<b>P</b> V <b>G</b> K <b>L</b> VL <b>N</b> R <b>N</b>	320	<b>303.20</b>	204.13 147.11
321	PANYFAEVEQ	MAFDPS <b>N</b> MP	GIEPSPD <b>K</b> ML	QGR <b>L</b> FAYPDT	HR <b>H</b> RLGPNYL	QIPV <b>N</b> CPY <b>R</b> A	<b>R</b> V <b>A</b> NY <b>Q</b> R <b>D</b> GP	<b>M</b> CM <b>H</b> DN <b>Q</b> GG <b>A</b>	400	623.31	<b>508.29</b> 345.22 288.20 175.12
401	PNYYPNSFSA	PEQQ <b>S</b> SA <b>L</b> EH	HS <b>Q</b> C <b>S</b> AD <b>V</b> K <b>R</b>	FNSANED <b>N</b> VT	QVR <b>T</b> FY <b>T</b> K <b>V</b> L	NEE <b>E</b> R <b>K</b> RL <b>C</b> E	NIAN <b>H</b> L <b>K</b> DA <b>Q</b>	<b>L</b> F <b>I</b> Q <b>R</b> K <b>A</b> V <b>K</b> N	480	981.50	834.44 <b>720.39</b> 623.34 476.27 <b>361.24</b> 248.16 147.11
481	<b>P</b> T <b>D</b> V <b>H</b> PDYGA	<b>R</b> VQ <b>A</b> LLDQYN	SQ <b>K</b> PK <b>N</b> AIHT	YVQAGSHIAA	<b>K</b> G <b>K</b> AN <b>L</b>				526	1805.93	1691.89 1505.81 <b>1390.78</b> 1277.70 1178.63 1121.61 1007.57

The matched peptides cover 12% (64/526 AA's) of the protein.

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078

# Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**  
 Peptide Mass MH<sup>+</sup>(Average): **1277.38**  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): **1276.6168**  
 Elemental Composition: [C55 H86 N15 O20](#)  
 Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**  
 Variable Modifications: **PTM - KMQSTY**  
 All fragment ion masses below are calculated as: **Monoisotopic masses**  
 (MH)<sup>+</sup>(Average): 639.1939  
 (MH)<sup>+</sup>(Monoisotopic): 638.8120

## N-terminal ions

b ions 114.0924 185.1296 313.1881 442.2307 557.2577 654.3104 769.3374 932.4007 989.4222 1102.5062

--- --- 157.0977<sup>+2</sup> 221.6190<sup>+2</sup> 279.1325<sup>+2</sup> 327.6589<sup>+2</sup> 385.1723<sup>+2</sup> 466.7040<sup>+2</sup> 495.2147<sup>+2</sup> 551.7568<sup>+2</sup>

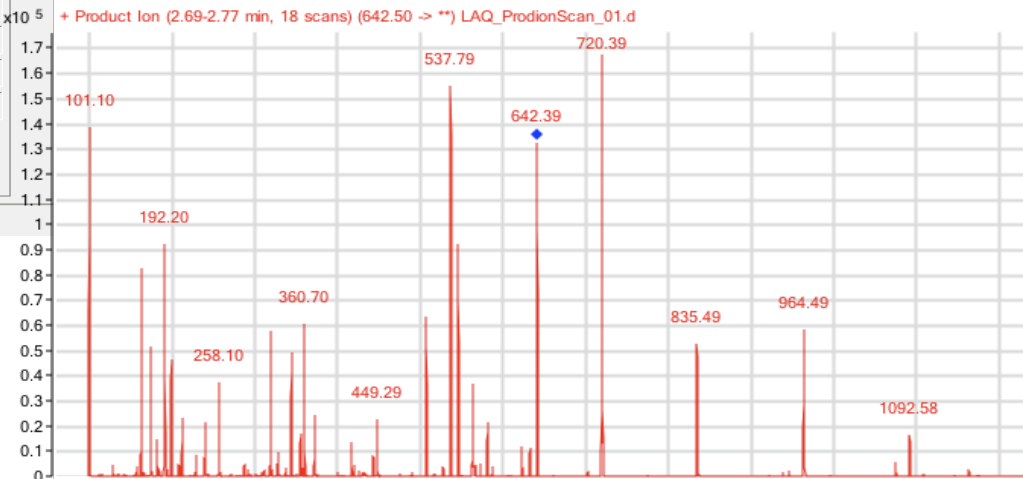
1 2 3 4 5 6 7 8 9 10 11  
 H - L A Q E D P D Y G L R -OH  
 11 10 9 8 7 6 5 4 3 2 1

## C-terminal ions

1163.5327 1092.4956 964.4371 835.3945 720.3675 623.3148 508.2878 345.2245 288.2030 175.1190  
 582.2700<sup>+2</sup> 546.7515<sup>+2</sup> 482.7222<sup>+2</sup> 418.2009<sup>+2</sup> 360.6874<sup>+2</sup> 312.1610<sup>+2</sup> 254.6475<sup>+2</sup> 173.1159<sup>+2</sup> 144.6051<sup>+2</sup> 88.0631<sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	88.0631 <sup>+2</sup>	y <sub>4</sub> <sup>+2</sup>	254.6475 <sup>+2</sup>	b <sub>7</sub> <sup>+2</sup>	385.1723 <sup>+2</sup>	b <sub>10</sub> <sup>+2</sup>	551.7568 <sup>+2</sup>	b <sub>8</sub>	932.4007
b <sub>1</sub>	114.0924	b <sub>5</sub> <sup>+2</sup>	279.1325 <sup>+2</sup>	y <sub>7</sub> <sup>+2</sup>	418.2009 <sup>+2</sup>	b <sub>5</sub>	557.2577	y <sub>8</sub>	964.4371
y <sub>2</sub> <sup>+2</sup>	144.6051 <sup>+2</sup>	y <sub>2</sub>	288.2030	b <sub>4</sub>	442.2307	y <sub>10</sub> <sup>+2</sup>	582.2700 <sup>+2</sup>	b <sub>9</sub>	989.4222
b <sub>3</sub> <sup>+2</sup>	157.0977 <sup>+2</sup>	y <sub>5</sub> <sup>+2</sup>	312.1610 <sup>+2</sup>	b <sub>8</sub> <sup>+2</sup>	466.7040 <sup>+2</sup>	y <sub>5</sub>	623.3148	y <sub>9</sub>	1092.4956
y <sub>3</sub> <sup>+2</sup>	173.1159 <sup>+2</sup>	b <sub>3</sub>	313.1881	y <sub>8</sub> <sup>+2</sup>	482.7222 <sup>+2</sup>	b <sub>6</sub>	654.3104	b <sub>10</sub>	1102.5062
y <sub>1</sub>	175.1190	b <sub>6</sub> <sup>+2</sup>	327.6589 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	495.2147 <sup>+2</sup>	y <sub>6</sub>	720.3675	y <sub>10</sub>	1163.5327
b <sub>2</sub>	185.1296	y <sub>3</sub>	345.2245	y <sub>4</sub>	508.2878	b <sub>7</sub>	769.3374		
b <sub>4</sub> <sup>+2</sup>	221.6190 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.6874 <sup>+2</sup>	y <sub>9</sub> <sup>+2</sup>	546.7515 <sup>+2</sup>	y <sub>7</sub>	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**  
 Peptide Mass MH<sup>+</sup>(Average): **1277.38**  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): **1276.6168**  
 Elemental Composition: **C56 H86 N15 O20**  
 Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**  
 Variable Modifications: **PTM - KMQSTY**  
 All fragment ion masses below are calculated as: **Monoisotopic masses**  
 (MH)<sup>+</sup>(Average): 639.1939  
 (MH)<sup>+</sup>(Monoisotopic): 638.8120

## N-terminal ions

b ions 114.0924 185.1296 313.1881 442.2307 557.2577 654.3104 769.3374 932.4007 989.4222 1102.5062

--- --- 157.0977<sup>+2</sup> 221.6190<sup>+2</sup> 279.1325<sup>+2</sup> 327.6589<sup>+2</sup> 385.1723<sup>+2</sup> 466.7040<sup>+2</sup> 495.2147<sup>+2</sup> 551.7568<sup>+2</sup>

1 2 3 4 5 6 7 8 9 10 11  
 H - L A Q E D P D Y G L R -OH  
 11 10 9 8 7 6 5 4 3 2 1

## C-terminal ions

1163.5327 1092.4956 964.4371 835.3945 720.3675 623.3148 508.2878 345.2245 288.2030 175.1190

582.2700<sup>+2</sup> 546.7515<sup>+2</sup> 482.7222<sup>+2</sup> 418.2009<sup>+2</sup> 360.6874<sup>+2</sup> 312.1610<sup>+2</sup> 254.6475<sup>+2</sup> 173.1159<sup>+2</sup> 144.6051<sup>+2</sup> 88.0631<sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	88.0631 <sup>+2</sup>	y <sub>4</sub> <sup>+2</sup>	254.6475 <sup>+2</sup>	b <sub>7</sub> <sup>+2</sup>	385.1723 <sup>+2</sup>	b <sub>10</sub> <sup>+2</sup>	551.7568 <sup>+2</sup>	b <sub>8</sub>	932.4007
b <sub>1</sub>	114.0924	b <sub>5</sub> <sup>+2</sup>	279.1325 <sup>+2</sup>	y <sub>7</sub> <sup>+2</sup>	418.2009 <sup>+2</sup>	b <sub>5</sub>	557.2577	y <sub>8</sub>	964.4371
y <sub>2</sub> <sup>+2</sup>	144.6051 <sup>+2</sup>	y <sub>2</sub>	288.2030	b <sub>4</sub>	442.2307	y <sub>10</sub> <sup>+2</sup>	582.2700 <sup>+2</sup>	b <sub>9</sub>	989.4222
b <sub>3</sub> <sup>+2</sup>	157.0977 <sup>+2</sup>	y <sub>5</sub> <sup>+2</sup>	312.1610 <sup>+2</sup>	b <sub>8</sub> <sup>+2</sup>	466.7040 <sup>+2</sup>	y <sub>5</sub>	623.3148	y <sub>9</sub>	1092.4956
y <sub>3</sub> <sup>+2</sup>	173.1159 <sup>+2</sup>	b <sub>3</sub>	313.1881	y <sub>8</sub> <sup>+2</sup>	482.7222 <sup>+2</sup>	b <sub>6</sub>	654.3104	b <sub>10</sub>	1102.5062
y <sub>1</sub>	175.1190	b <sub>6</sub> <sup>+2</sup>	327.6589 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	495.2147 <sup>+2</sup>	y <sub>6</sub>	720.3675	y <sub>10</sub>	1163.5327
b <sub>2</sub>	185.1296	y <sub>3</sub>	345.2245	y <sub>4</sub>	508.2878	b <sub>7</sub>	769.3374		
b <sub>4</sub> <sup>+2</sup>	221.6190 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.6874 <sup>+2</sup>	y <sub>9</sub> <sup>+2</sup>	546.7515 <sup>+2</sup>	y <sub>7</sub>	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**  
 Peptide Mass MH<sup>+</sup>(Average): **1277.38**  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): **1276.6168**  
 Elemental Composition: [C56 H86 N15 O20](#)  
 Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**  
 Variable Modifications: **PTM - KMQSTY**  
 All fragment ion masses below are calculated as: **Monoisotopic masses**  
 (MH)<sup>+</sup>2(Average): 639.1939  
 (MH)<sup>+</sup>2(Monoisotopic): 638.8120

## N-terminal ions

b ions 114.0924 185.1296 313.1881 442.2307 557.2577 654.3104 769.3374 932.4007 989.4222 1102.5062

--- --- 157.0977<sup>+2</sup> 221.6190<sup>+2</sup> 279.1325<sup>+2</sup> 327.6589<sup>+2</sup> 385.1723<sup>+2</sup> 466.7040<sup>+2</sup> 495.2147<sup>+2</sup> 551.7568<sup>+2</sup>

1 2 3 4 5 6 7 8 9 10 11  
 H - L A Q E D P D Y G L R -OH  
 11 10 9 8 7 6 5 4 3 2 1

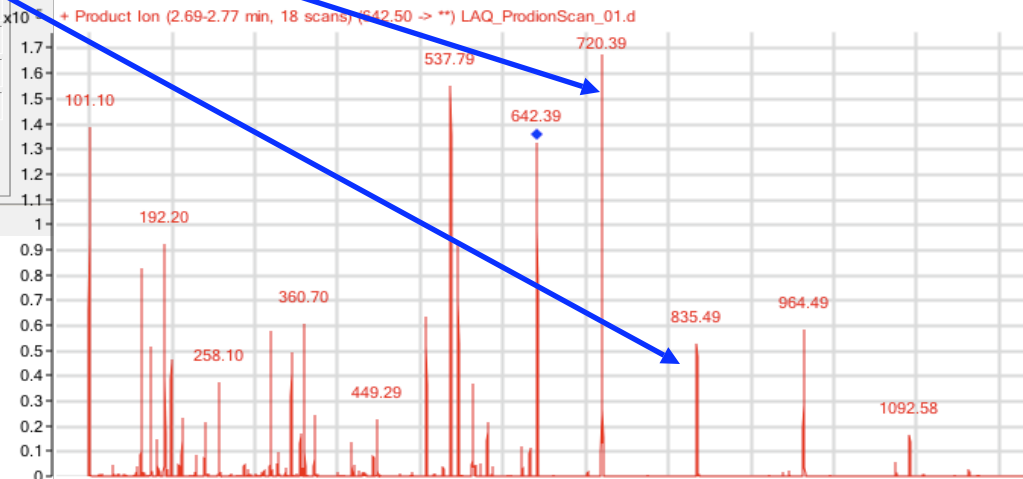
## C-terminal ions

1163.5327 1092.4956 964.4371 835.3945 720.3675 623.3148 508.2878 345.2245 288.2030 175.1190

582.2700<sup>+2</sup> 546.7515<sup>+2</sup> 482.7222<sup>+2</sup> 418.2009<sup>+2</sup> 360.6874<sup>+2</sup> 312.1610<sup>+2</sup> 254.6475<sup>+2</sup> 173.1159<sup>+2</sup> 144.6051<sup>+2</sup> 88.0631<sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	88.0631 <sup>+2</sup>	y <sub>4</sub> <sup>+2</sup>	254.6475 <sup>+2</sup>	b <sub>7</sub> <sup>+2</sup>	385.1723 <sup>+2</sup>	b <sub>10</sub> <sup>+2</sup>	551.7568 <sup>+2</sup>	b <sub>8</sub>	932.4007
b <sub>1</sub>	114.0924	b <sub>5</sub> <sup>+2</sup>	279.1325 <sup>+2</sup>	y <sub>7</sub> <sup>+2</sup>	418.2009 <sup>+2</sup>	b <sub>5</sub>	557.2577	y <sub>8</sub>	964.4371
y <sub>2</sub> <sup>+2</sup>	144.6051 <sup>+2</sup>	y <sub>2</sub>	288.2030	b <sub>4</sub>	442.2307	y <sub>10</sub> <sup>+2</sup>	582.2700 <sup>+2</sup>	b <sub>9</sub>	989.4222
b <sub>3</sub> <sup>+2</sup>	157.0977 <sup>+2</sup>	y <sub>5</sub> <sup>+2</sup>	312.1610 <sup>+2</sup>	b <sub>8</sub> <sup>+2</sup>	466.7040 <sup>+2</sup>	y <sub>5</sub>	623.3148	y <sub>9</sub>	1092.4956
y <sub>3</sub> <sup>+2</sup>	173.1159 <sup>+2</sup>	b <sub>3</sub>	313.1881	y <sub>8</sub> <sup>+2</sup>	482.7222 <sup>+2</sup>	b <sub>6</sub>	654.3104	b <sub>10</sub>	1102.5062
y <sub>1</sub>	175.1190	b <sub>6</sub> <sup>+2</sup>	327.6589 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	495.2147 <sup>+2</sup>	y <sub>6</sub>	720.3675	y <sub>10</sub>	1163.5327
b <sub>2</sub>	185.1296	y <sub>3</sub>	345.2245	y <sub>4</sub>	508.2878	b <sub>7</sub>	769.3374		
b <sub>4</sub> <sup>+2</sup>	221.6190 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.6874 <sup>+2</sup>	y <sub>9</sub> <sup>+2</sup>	546.7515 <sup>+2</sup>	y <sub>7</sub>	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**  
 Peptide Mass MH<sup>+</sup>(Average): **1277.38**  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): **1276.6168**  
 Elemental Composition: [C55 H86 N15 O20](#)  
 Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**  
 Variable Modifications: **PTM - KMQSTY**  
 All fragment ion masses below are calculated as: **Monoisotopic masses**  
 (MH)<sup>+</sup>(Average): 639.1939  
 (MH)<sup>+</sup>(Monoisotopic): 638.8120

## N-terminal ions

b ions 114.0924 185.1296 313.1881 442.2307 557.2577 654.3104 769.3374 932.4007 989.4222 1102.5062

--- --- 157.0977<sup>+2</sup> 221.6190<sup>+2</sup> 279.1325<sup>+2</sup> 327.6589<sup>+2</sup> 385.1723<sup>+2</sup> 466.7040<sup>+2</sup> 495.2147<sup>+2</sup> 551.7568<sup>+2</sup>

1 2 3 4 5 6 7 8 9 10 11  
 H - L A Q E D P D Y G L R -OH  
 11 10 9 8 7 6 5 4 3 2 1

## C-terminal ions

1163.5327 1092.4956 964.4371 835.3945 720.3675 623.3148 508.2878 345.2245 288.2030 175.1190

582.2700<sup>+2</sup> 546.7515<sup>+2</sup> 482.7222<sup>+2</sup> 418.2009<sup>+2</sup> 360.6874<sup>+2</sup> 312.1610<sup>+2</sup> 254.6475<sup>+2</sup> 173.1159<sup>+2</sup> 144.6051<sup>+2</sup> 88.0631<sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	88.0631 <sup>+2</sup>	y <sub>4</sub> <sup>+2</sup>	254.6475 <sup>+2</sup>	b <sub>7</sub> <sup>+2</sup>	385.1723 <sup>+2</sup>	b <sub>10</sub> <sup>+2</sup>	557.2577 <sup>+2</sup>	b <sub>8</sub>	932.4007
b <sub>1</sub>	114.0924	b <sub>5</sub> <sup>+2</sup>	279.1325 <sup>+2</sup>	y <sub>7</sub> <sup>+2</sup>	418.2009 <sup>+2</sup>	b <sub>5</sub>	557.2577	y <sub>8</sub>	964.4371
y <sub>2</sub> <sup>+2</sup>	144.6051 <sup>+2</sup>	y <sub>2</sub>	288.2030	b <sub>4</sub>	442.2307	y <sub>10</sub> <sup>+2</sup>	582.2700 <sup>+2</sup>	b <sub>9</sub>	989.4222
b <sub>3</sub> <sup>+2</sup>	157.0977 <sup>+2</sup>	y <sub>5</sub> <sup>+2</sup>	312.1610 <sup>+2</sup>	b <sub>8</sub> <sup>+2</sup>	466.7040 <sup>+2</sup>	y <sub>5</sub>	623.3148	y <sub>9</sub>	1092.4956
y <sub>3</sub> <sup>+2</sup>	173.1159 <sup>+2</sup>	b <sub>3</sub>	313.1881	y <sub>8</sub> <sup>+2</sup>	482.7222 <sup>+2</sup>	b <sub>6</sub>	654.3104	b <sub>10</sub>	1102.5062
y <sub>1</sub>	175.1190	b <sub>6</sub> <sup>+2</sup>	327.6589 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	495.2147 <sup>+2</sup>	y <sub>6</sub>	720.3675	y <sub>10</sub>	1163.5327
b <sub>2</sub>	185.1296	y <sub>3</sub>	345.2245	y <sub>4</sub>	508.2878	b <sub>7</sub>	769.3374		
b <sub>4</sub> <sup>+2</sup>	221.6190 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.6874 <sup>+2</sup>	y <sub>9</sub> <sup>+2</sup>	546.7515 <sup>+2</sup>	y <sub>7</sub>	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**  
 Peptide Mass MH<sup>+</sup>(Average): **1277.38**  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): **1276.6168**  
 Elemental Composition: [C55 H86 N15 O20](#)  
 Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**  
 Variable Modifications: **PTM - KMQSTY**  
 All fragment ion masses below are calculated as: **Monoisotopic masses**  
 (MH)<sup>+</sup>2(Average): 639.1939  
 (MH)<sup>+</sup>2(Monoisotopic): 638.8120

## N-terminal ions

b ions 114.0924 185.1296 313.1881 442.2307 557.2577 654.3104 769.3374 932.4007 989.4222 1102.5062

--- --- 157.0977<sup>+2</sup> 221.6190<sup>+2</sup> 279.1325<sup>+2</sup> 327.6589<sup>+2</sup> 385.1723<sup>+2</sup> 466.7040<sup>+2</sup> 495.2147<sup>+2</sup> 551.7568<sup>+2</sup>

1 2 3 4 5 6 7 8 9 10 11  
 H - L A Q E D P D Y G L R -OH  
 11 10 9 8 7 6 5 4 3 2 1

## C-terminal ions

1163.5327 1092.4956 964.4371 835.3945 720.3675 623.3148 508.2878 345.2245 288.2030 175.1190

582.2700<sup>+2</sup> 546.7515<sup>+2</sup> 482.7222<sup>+2</sup> 418.2009<sup>+2</sup> 360.6874<sup>+2</sup> 312.1610<sup>+2</sup> 254.6475<sup>+2</sup> 173.1159<sup>+2</sup> 144.6051<sup>+2</sup> 88.0631<sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	88.0631 <sup>+2</sup>	y <sub>4</sub> <sup>+2</sup>	254.6475 <sup>+2</sup>	b <sub>7</sub> <sup>+2</sup>	385.1723 <sup>+2</sup>	b <sub>10</sub> <sup>+2</sup>	557.2577 <sup>+2</sup>	b <sub>8</sub>	932.4007
b <sub>1</sub>	114.0924	b <sub>5</sub> <sup>+2</sup>	279.1325 <sup>+2</sup>	y <sub>7</sub> <sup>+2</sup>	418.2009 <sup>+2</sup>	b <sub>5</sub>	557.2577	y <sub>8</sub>	964.4371
y <sub>2</sub> <sup>+2</sup>	144.6051 <sup>+2</sup>	y <sub>2</sub>	288.2030	b <sub>4</sub>	442.2307	y <sub>10</sub> <sup>+2</sup>	582.2700 <sup>+2</sup>	b <sub>9</sub>	989.4222
b <sub>3</sub> <sup>+2</sup>	157.0977 <sup>+2</sup>	y <sub>5</sub> <sup>+2</sup>	312.1610 <sup>+2</sup>	b <sub>8</sub> <sup>+2</sup>	466.7040 <sup>+2</sup>	y <sub>5</sub>	623.3148	y <sub>9</sub>	1092.4956
y <sub>3</sub> <sup>+2</sup>	173.1159 <sup>+2</sup>	b <sub>3</sub>	313.1881	y <sub>8</sub> <sup>+2</sup>	482.7222 <sup>+2</sup>	b <sub>6</sub>	654.3104	b <sub>10</sub>	1102.5062
y <sub>1</sub>	175.1190	b <sub>6</sub> <sup>+2</sup>	327.6589 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	495.2147 <sup>+2</sup>	y <sub>6</sub>	720.3675	y <sub>10</sub>	1163.5327
b <sub>2</sub>	185.1296	y <sub>3</sub>	345.2245	y <sub>4</sub>	508.2878	b <sub>7</sub>	769.3374		
b <sub>4</sub> <sup>+2</sup>	221.6190 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.6874 <sup>+2</sup>	y <sub>9</sub> <sup>+2</sup>	546.7515 <sup>+2</sup>	y <sub>7</sub>	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**  
 Peptide Mass MH<sup>+</sup>(Average): 1277.38  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1276.6168  
 Elemental Composition: [C55 H86 N15 O20](#)  
 Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**  
 Variable Modifications: **PTM - KMQSTY**  
 All fragment ion masses below are calculated as: **Monoisotopic masses**  
 (MH)<sup>+</sup>2(Average): 639.1939  
 (MH)<sup>+</sup>2(Monoisotopic): 638.8120

## N-terminal ions

b ions 114.0924 185.1296 313.1881 442.2307 557.2577 654.3104 769.3374 932.4007 989.4222 1102.5062

--- --- 157.0977<sup>+2</sup> 221.6190<sup>+2</sup> 279.1325<sup>+2</sup> 327.6589<sup>+2</sup> 385.1723<sup>+2</sup> 466.7040<sup>+2</sup> 495.2147<sup>+2</sup> 551.7568<sup>+2</sup>

1 2 3 4 5 6 7 8 9 10 11  
 H - L A Q E D P D Y G L R -OH  
 11 10 9 8 7 6 5 4 3 2 1

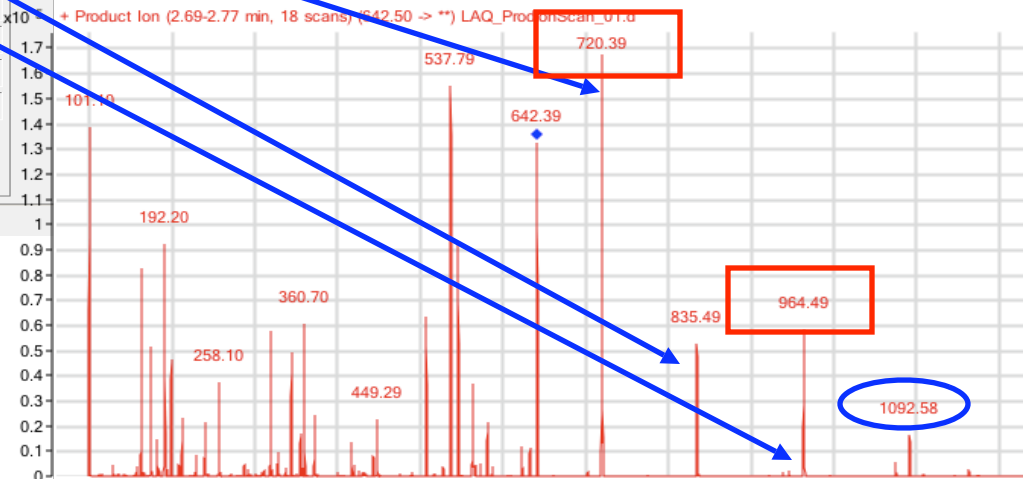
## C-terminal ions

1163.5327 1092.4956 964.4371 835.3945 720.3675 623.3148 508.2878 345.2245 288.2030 175.1190

582.2700<sup>+2</sup> 546.7515<sup>+2</sup> 482.7222<sup>+2</sup> 418.2009<sup>+2</sup> 360.6874<sup>+2</sup> 312.1610<sup>+2</sup> 254.6475<sup>+2</sup> 173.1159<sup>+2</sup> 144.6051<sup>+2</sup> 88.0631<sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	88.0631 <sup>+2</sup>	y <sub>4</sub> <sup>+2</sup>	254.6475 <sup>+2</sup>	b <sub>7</sub> <sup>+2</sup>	385.1723 <sup>+2</sup>	b <sub>10</sub> <sup>+2</sup>	557.2577 <sup>+2</sup>	b <sub>8</sub>	932.4007
b <sub>1</sub>	114.0924	b <sub>5</sub> <sup>+2</sup>	279.1325 <sup>+2</sup>	y <sub>7</sub> <sup>+2</sup>	418.2009 <sup>+2</sup>	b <sub>5</sub>	557.2577	y <sub>8</sub>	964.4371
y <sub>2</sub> <sup>+2</sup>	144.6051 <sup>+2</sup>	y <sub>2</sub>	288.2030	b <sub>4</sub>	442.2307	y <sub>10</sub> <sup>+2</sup>	582.2700 <sup>+2</sup>	b <sub>9</sub>	989.4222
b <sub>3</sub> <sup>+2</sup>	157.0977 <sup>+2</sup>	y <sub>5</sub> <sup>+2</sup>	312.1610 <sup>+2</sup>	b <sub>8</sub> <sup>+2</sup>	466.7040 <sup>+2</sup>	y <sub>5</sub>	623.3148	y <sub>9</sub>	1092.4956
y <sub>3</sub> <sup>+2</sup>	173.1159 <sup>+2</sup>	b <sub>3</sub>	313.1881	y <sub>8</sub> <sup>+2</sup>	482.7222 <sup>+2</sup>	b <sub>6</sub>	654.3104	b <sub>10</sub>	1102.5062
y <sub>1</sub>	175.1190	b <sub>6</sub> <sup>+2</sup>	327.6589 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	495.2147 <sup>+2</sup>	y <sub>6</sub>	720.3675	y <sub>10</sub>	1163.5327
b <sub>2</sub>	185.1296	y <sub>3</sub>	345.2245	y <sub>4</sub>	508.2878	b <sub>7</sub>	769.3374		
b <sub>4</sub> <sup>+2</sup>	221.6190 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.6874 <sup>+2</sup>	y <sub>9</sub> <sup>+2</sup>	546.7515 <sup>+2</sup>	y <sub>7</sub>	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide EAE – Peptide Selector

Peptide Sequence: EAETFFPNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: C78 H111 N16 O24  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>2+</sup>(Average): 828.9225  
 (MH)<sup>2+</sup>(Monoisotopic): 828.4012

**N-terminal ions**

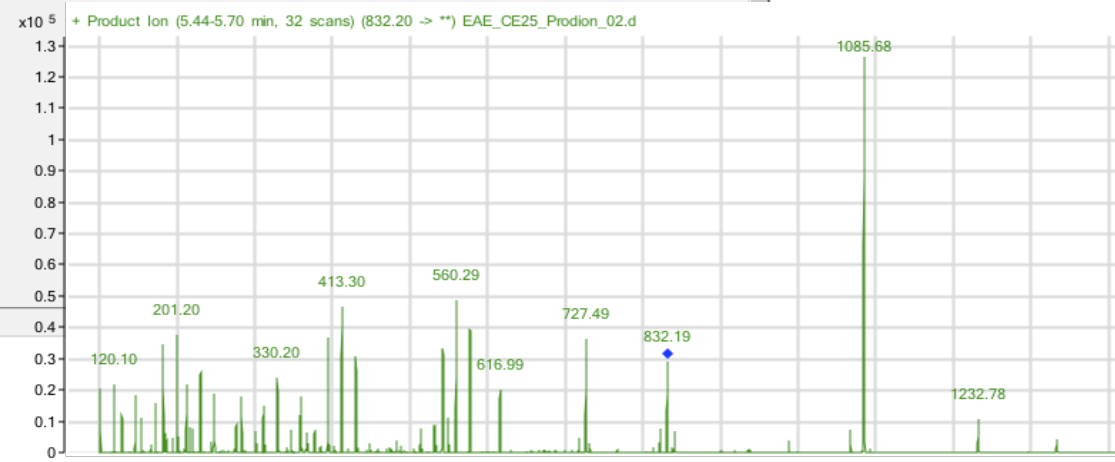
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907			
	---	---	---	---	---	---	---	468.7091 <sup>+2</sup>	517.2355 <sup>+2</sup>	590.7697 <sup>+2</sup>	648.2831 <sup>+2</sup>	704.8252 <sup>+2</sup>	755.3490 <sup>+2</sup>			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
	H -	E	A	E	T	F	P	F	N	P	F	D	L	T	K	-OH
	14	13	12	11	10	9	8	7	6	5	4	3	2	1		

**C-terminal ions**

y ions	1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128
	763.8799 <sup>+2</sup>	728.3614 <sup>+2</sup>	663.8401 <sup>+2</sup>	613.3162 <sup>+2</sup>	539.7820 <sup>+2</sup>	491.2556 <sup>+2</sup>	417.7214 <sup>+2</sup>	360.7000 <sup>+2</sup>	312.1736 <sup>+2</sup>	238.6394 <sup>+2</sup>	181.1259 <sup>+2</sup>	124.5839 <sup>+2</sup>	74.0600 <sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>9</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

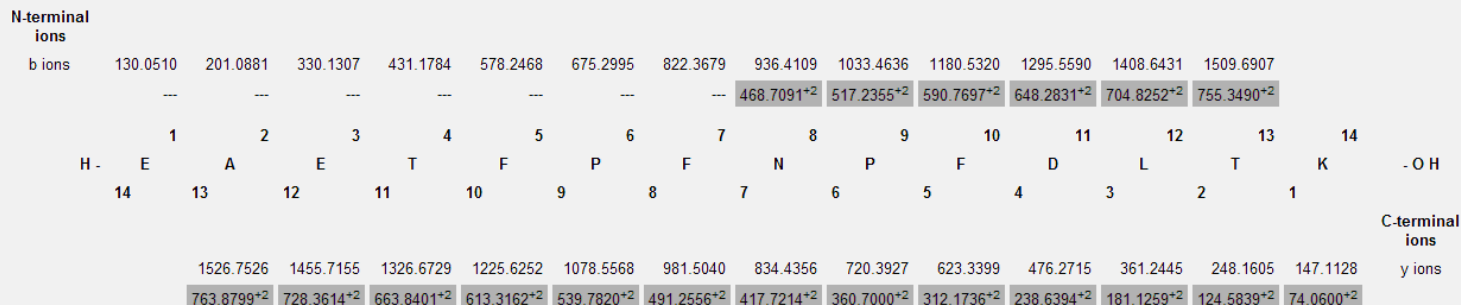
MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide EAE – Peptide Selector

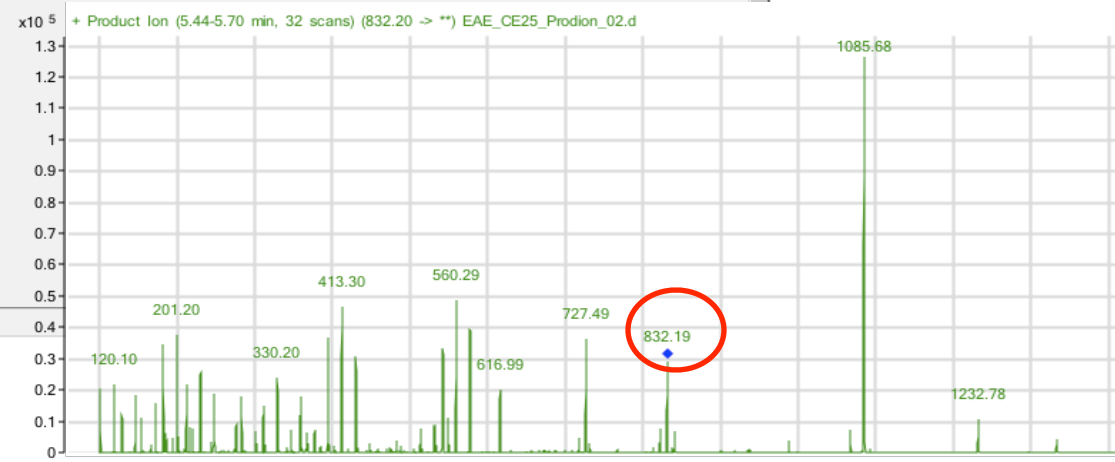
Peptide Sequence: EAETFFNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: C78 H111 N16 O24  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>2+</sup>(Average): 828.9225  
 (MH)<sup>2+</sup>(Monoisotopic): 828.4012



y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>9</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

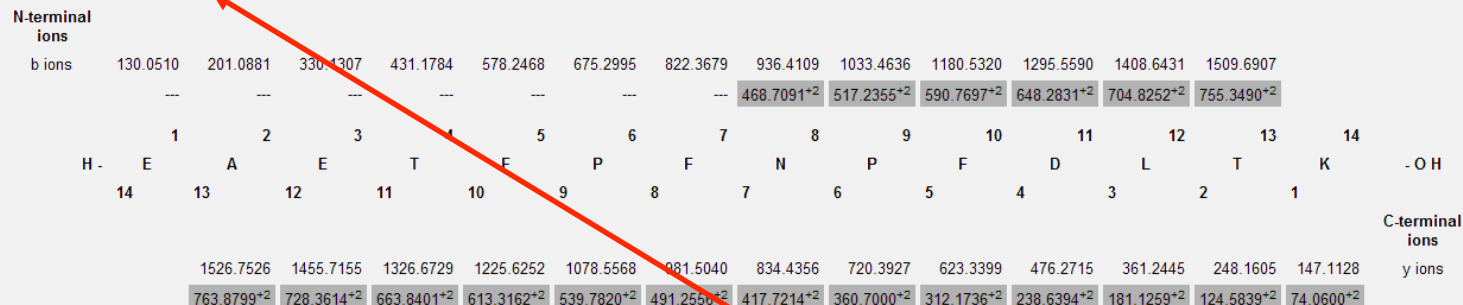
MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide EAE – Peptide Selector

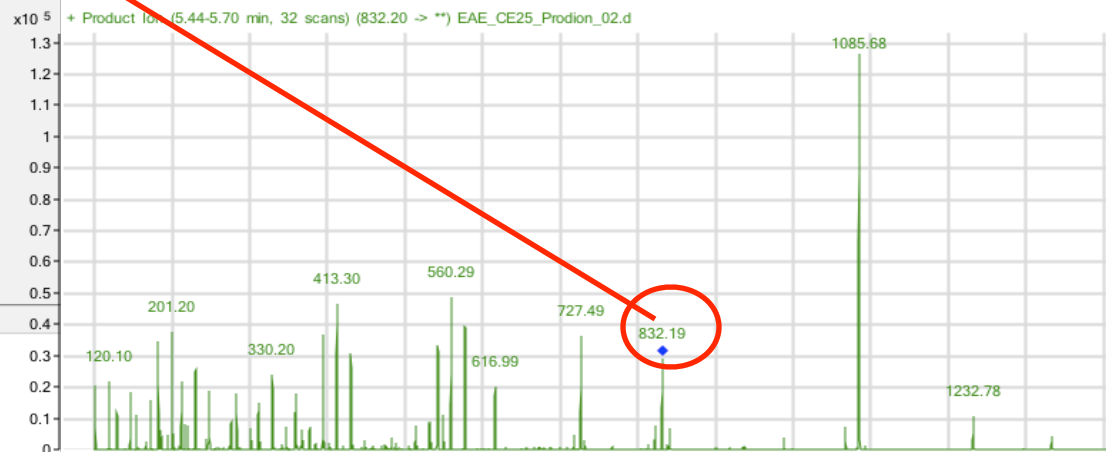
Peptide Sequence: EAETFPNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: C78 H111 N16 O24  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>2+</sup>(Average): 828.9225  
 (MH)<sup>2+</sup>(Monoisotopic): 828.4012



y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>5</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

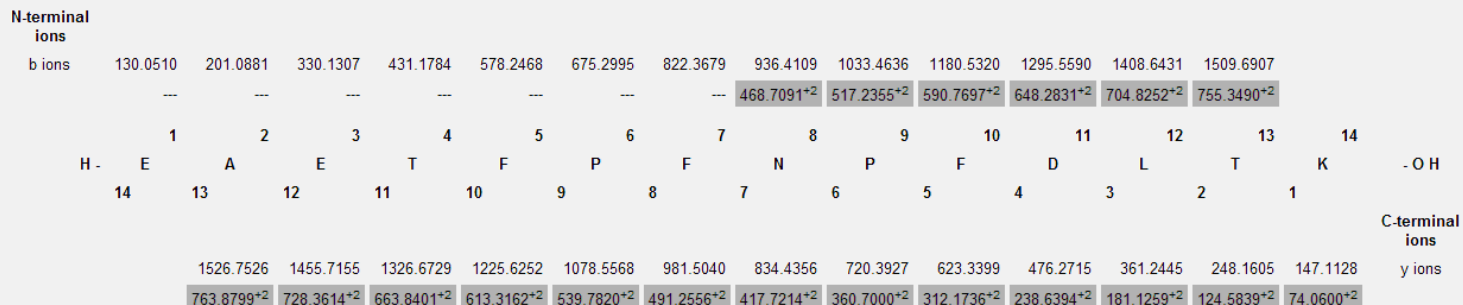
MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide EAE – Peptide Selector

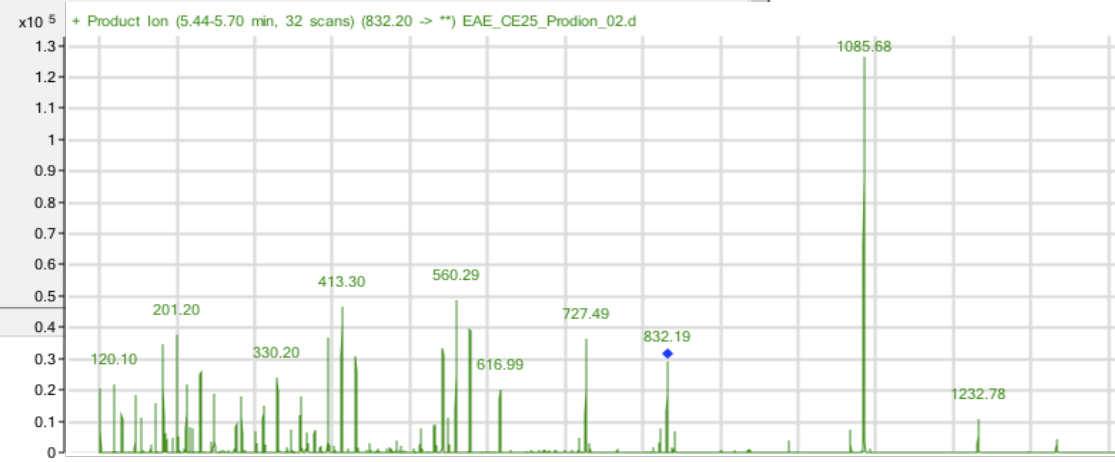
Peptide Sequence: EAETFFPNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: C78 H111 N16 O24  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>+</sup>(Average): 828.9225  
 (MH)<sup>+</sup>(Monoisotopic): 828.4012



y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>9</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

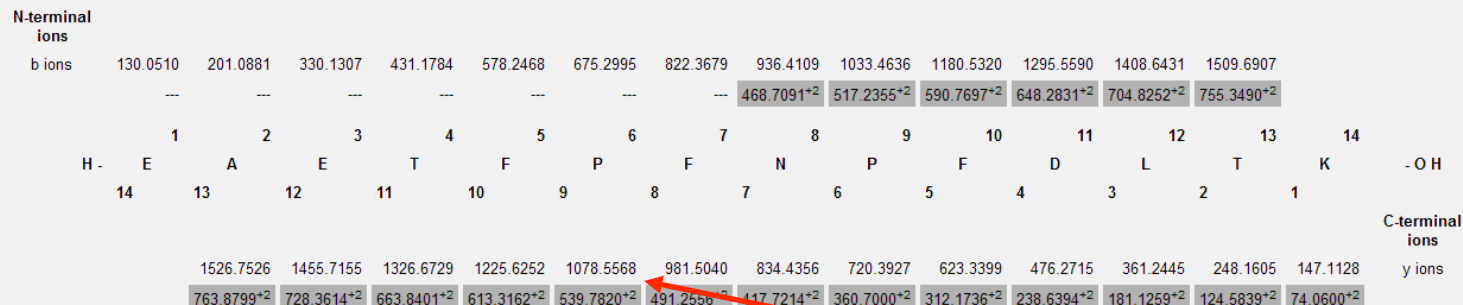
MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide EAE – Peptide Selector

Peptide Sequence: EAETFFNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: C78 H111 N16 O24  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>2+</sup>(Average): 828.9225  
 (MH)<sup>2+</sup>(Monoisotopic): 828.4012



y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>9</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

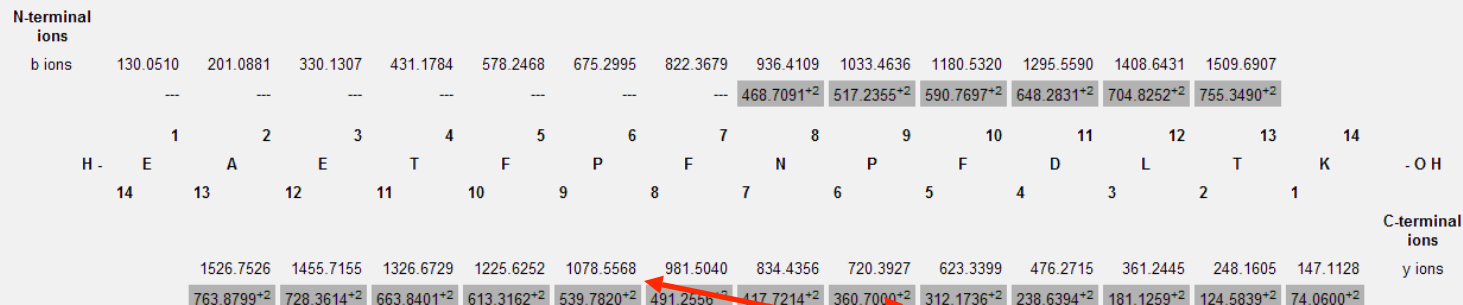
MS Product - Agilent Spectrum Mill Rev. 3.3.078



# Catalase Peptide EAE – Peptide Selector

Peptide Sequence: EAETFFNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: C78 H111 N16 O24  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>2+</sup>(Average): 828.9225  
 (MH)<sup>2+</sup>(Monoisotopic): 828.4012



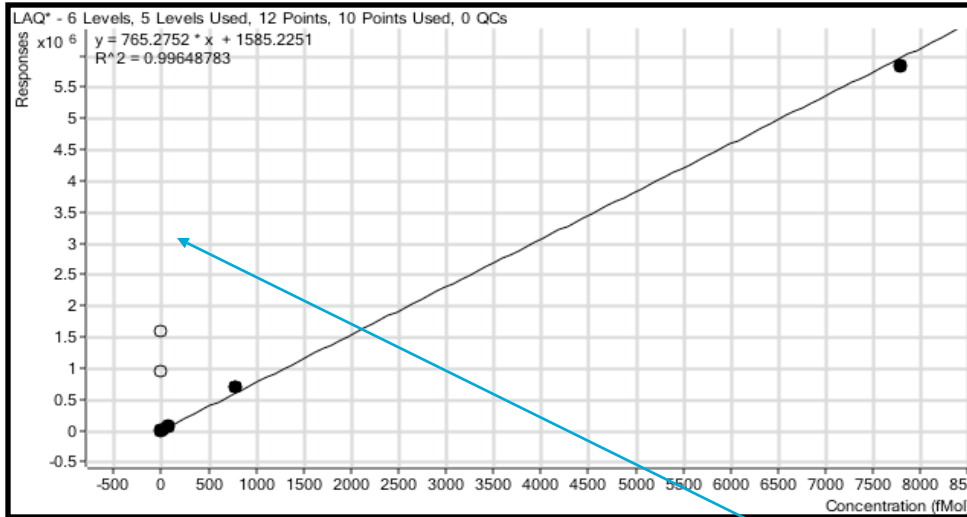
y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>9</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078



# External Calibration on Catalase Peptides

## Linearity : five order of magnitude

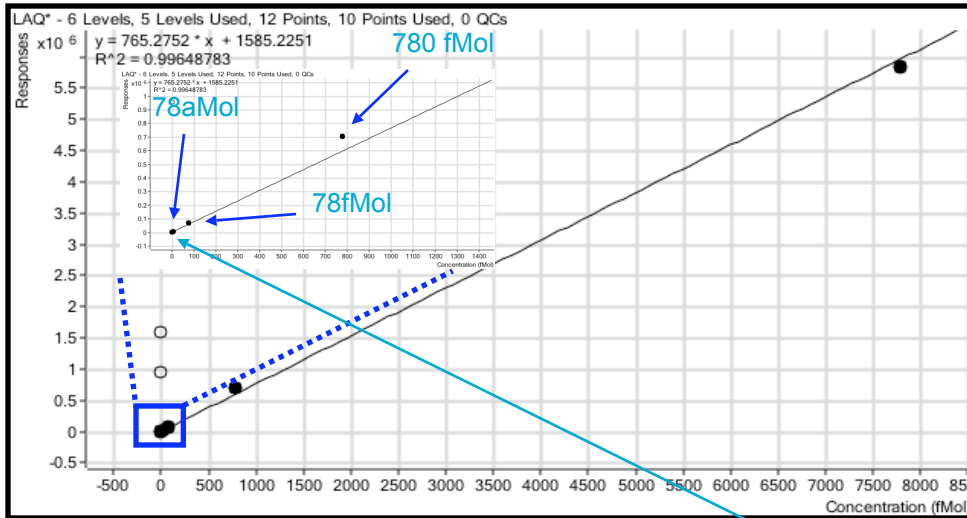


External quantitation curve  
of catalase peptide  
L\* AQEDPDYGLR from 78  
amol to 7800 fmol

RSD < 6%

# External Calibration on Catalase Peptides

## Linearity : five order of magnitude

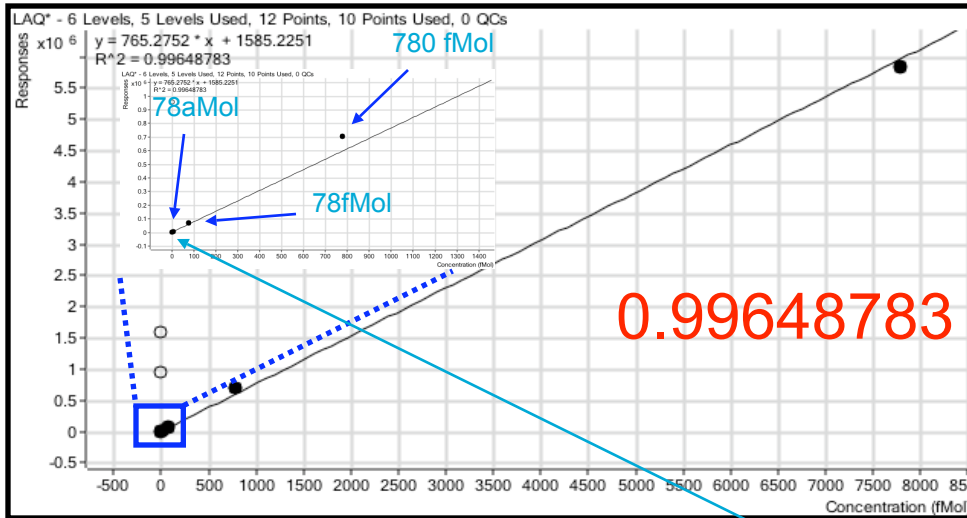


External quantitation curve of catalase peptide L\* AQEDPDYGLR from 78 amol to 7800 fmol

RSD < 6%

# External Calibration on Catalase Peptides

## Linearity : five order of magnitude

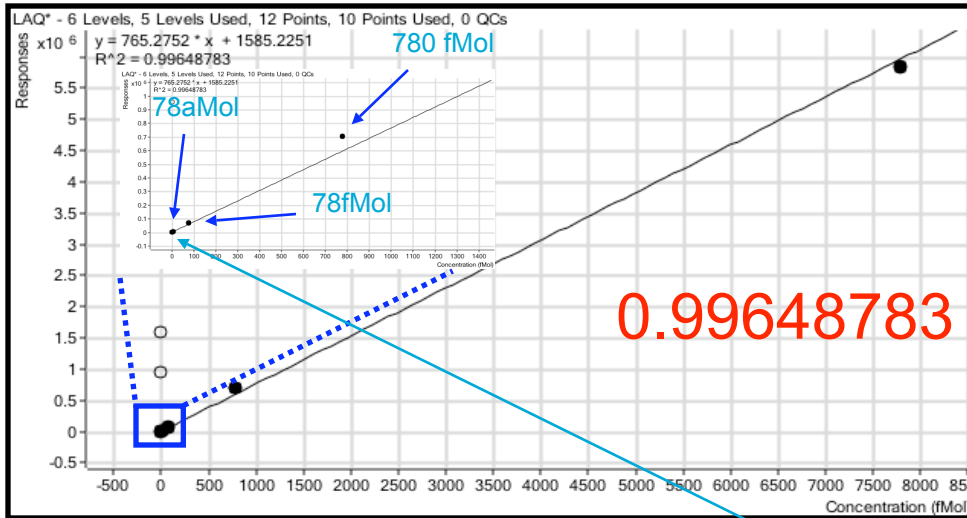


External quantitation curve  
of catalase peptide  
L\*AQEDPDYGLR from 78  
amol to 7800 fmol

RSD < 6%

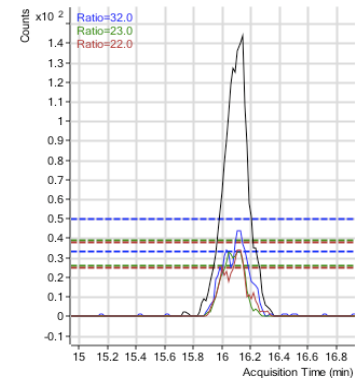
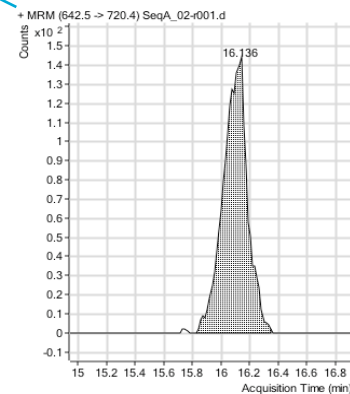
# External Calibration on Catalase Peptides

## Linearity : five order of magnitude



External quantitation curve of catalase peptide L\* AQEDPDYGLR from 78 amol to 7800 fmol

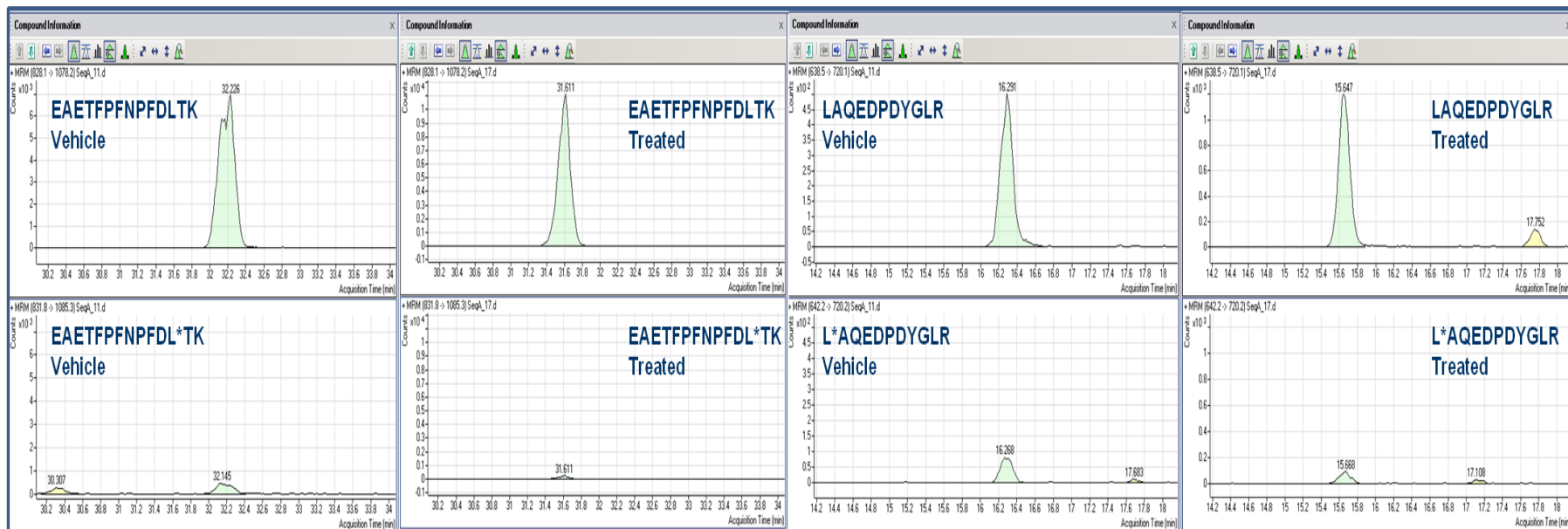
RSD < 6%



# Catalase Quantitation Results

Sample					EAE Met...	EAE Results							Qualifier (828.1 -> 720.1) Results			EAE* (ISTD) Results		Qualifier (831.8 -> 727.3) Results			
⚠	⚙	Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
		RL_Veh_1ppb	Cal	1	2/27/2008 7:49 AM		32.226	85633	169.81		14.6295	14.6295		10.9	44.30		32.145	5853	20.6	2.71	
		RL_HDose_1ppb	Cal	1	2/27/2008 3:09 PM		31.611	104655	298.12		54.3647	54.3647		10.6	71.79		31.611	1925	11.2	1.03	

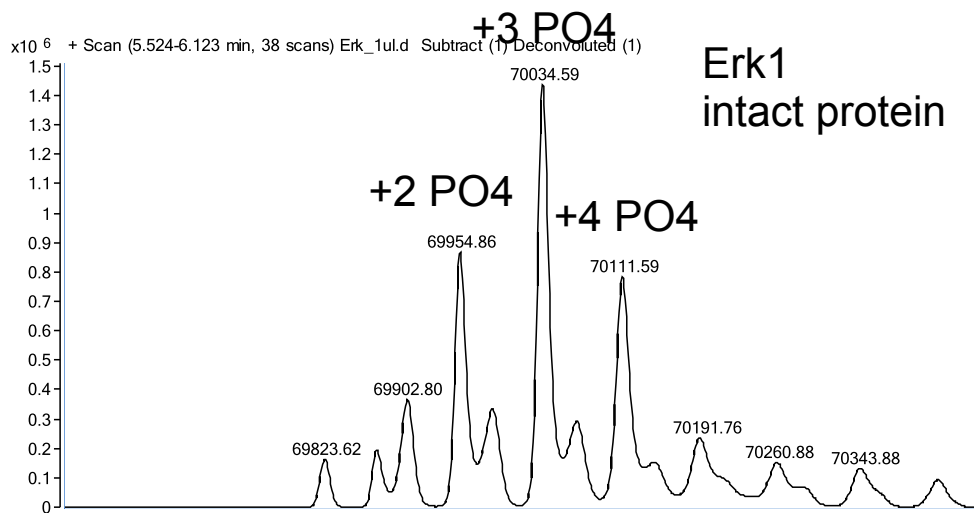
Sample					LAQ Met...	LAQ Results							Qualifier (638.5 -> 964.2) Results			LAQ* (ISTD) Results		Qualifier (642.2 -> 964.2) Results			
⚠	⚙	Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
		RL_Veh_1ppb	Cal	1	2/27/2008 7:49 AM		16.291	4781	71.89		5.8898	5.8898		55.4	35.63		16.268	812	49.9	13.65	
		RL_HDose_1ppb	Cal	1	2/27/2008 3:09 PM		15.647	10560	77.02		12.9958	12.9958		51.2	50.83		15.668	813	44.1	6.34	



# Catalase Quantitation Results

Sample	Peptide	Catalase (fmol/ug protein)	Catalase (pg/ug protein)	Fold Change EAETFPFNP FDLTK	Fold Change LAQEDPDY GLR	Fold Change 2D-DIGE
Vehicle Treated	EAETFPFNP FDLTK	8.84	14.63	1.00	1.00	1.00
	LAQEDPDYGL R	4.61	5.89			
Troglitazone Treated	EAETFPFNP FDLTK	32.69	54.36	3.7	2.2	1.45
	LAQEDPDYGL R	10.13	13.00			

# Quantitation of protein phosphorylation using MRM



# Selection of MRM transitions

TY: IADPEHDHTGFLTEY**V**ATR

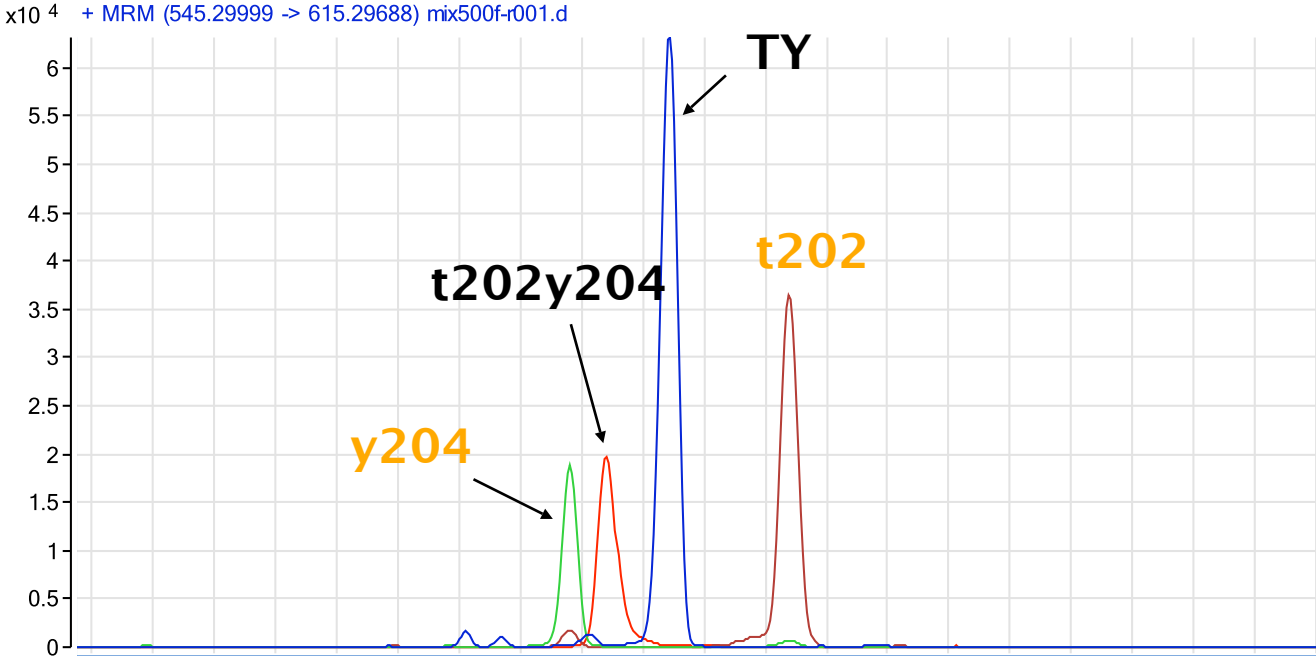
t202: IADPEHDHTGFLTEY**V**ATR

y204: IADPEHDHTGFLTEY**V**ATR

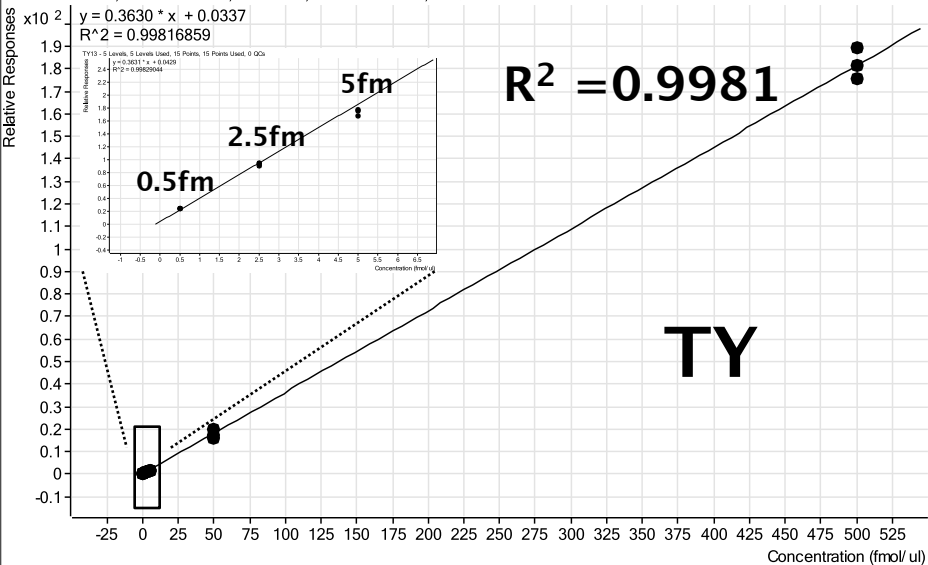
t202y204: IADPEHDHTGFLTEY**V**ATR

	Precursor ion	Product ions	
TY	545.3	615.3	782.5
	$[M+3H]^{3+}$	$y_5$	$b_{14}^{2+}$
t202	<u>753.3</u>	615.3	<u>979.9</u>
	$[M+2H]^{2+}$	$y_5$	$y_{16}^{2+}$
y204	<u>753.3</u>	<u>979.9</u>	695.3
	$[M+2H]^{2+}$	$y_{16}^{2+}$	$y_5$
t202y204 4	780.0	647.6	695.3
	$[M+2H]^{2+}$	$y_{16}^{3+-}$ $H_3PO_4$	$y_5$

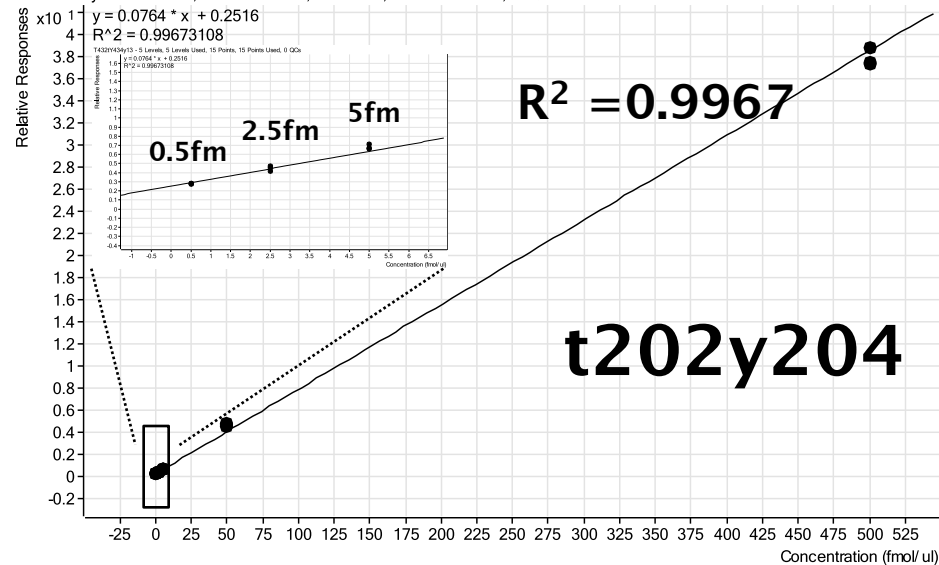
# Chromatographic Separation of the Four Peptide Standards allowed the selection of the same Q1 and Q3 transitions for two different peptides



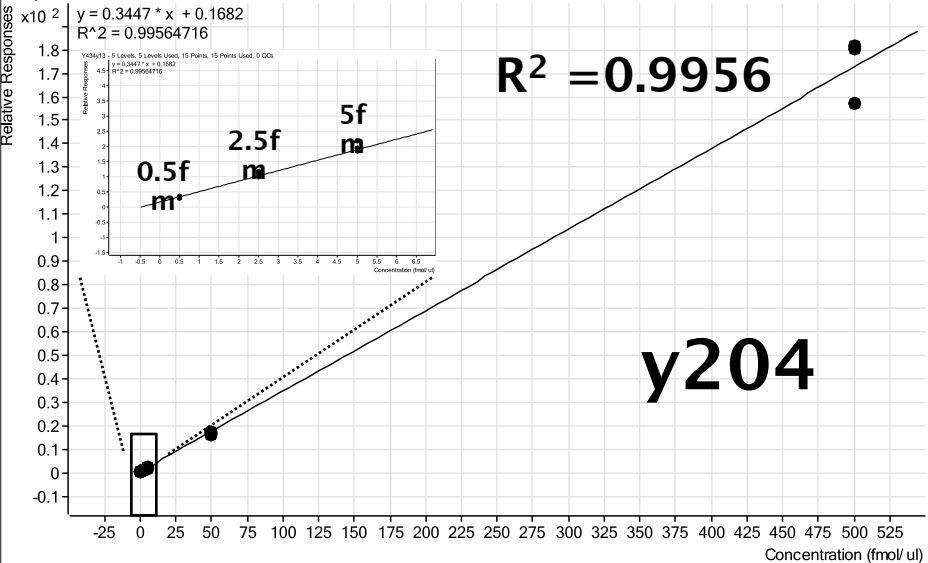
TY13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs



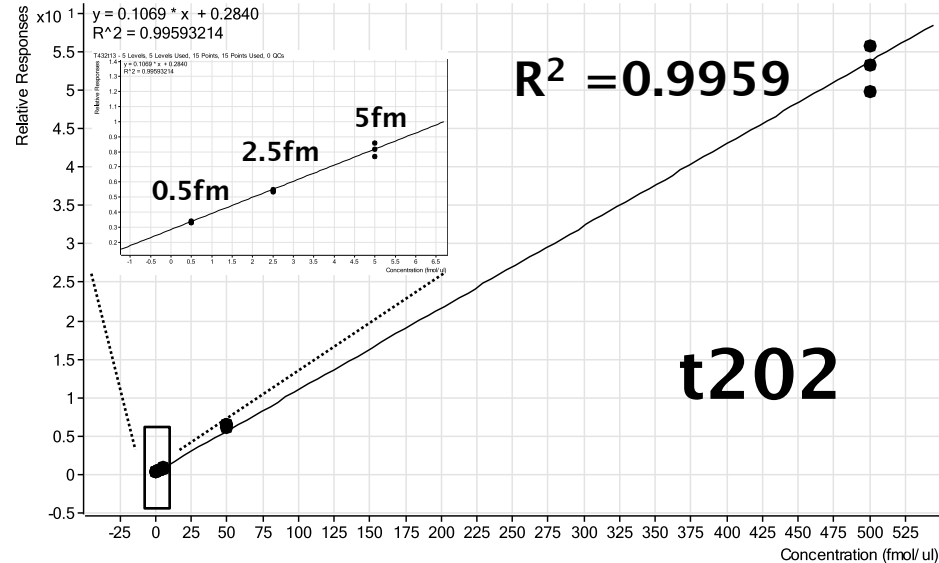
T432tY434y13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs



Y434y13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs



T432t13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs



# Quantitation of the degree of phosphorylation at T202 and Y204 in active Erk1 protein

peptide	% Molar ratio	RSD (n=9)
TY	<b>20%</b>	<b>0.13</b>
t202	<b>25%</b>	<b>0.15</b>
y204	<b>21%</b>	<b>0.12</b>
t202y204	<b>34%</b>	<b>0.08</b>

In this batch of active Erk1 sample, 59% of T202 and 55% of Y204 were phosphorylated

# HPLC-Chip/MS System

- Provide high sensitivity and large dynamic range
- Robust and stable nanoflow with HPLC-Chip
- Good retention time and MS detection reproducibility
- Peptide selector helps choosing MRM transitions