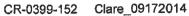
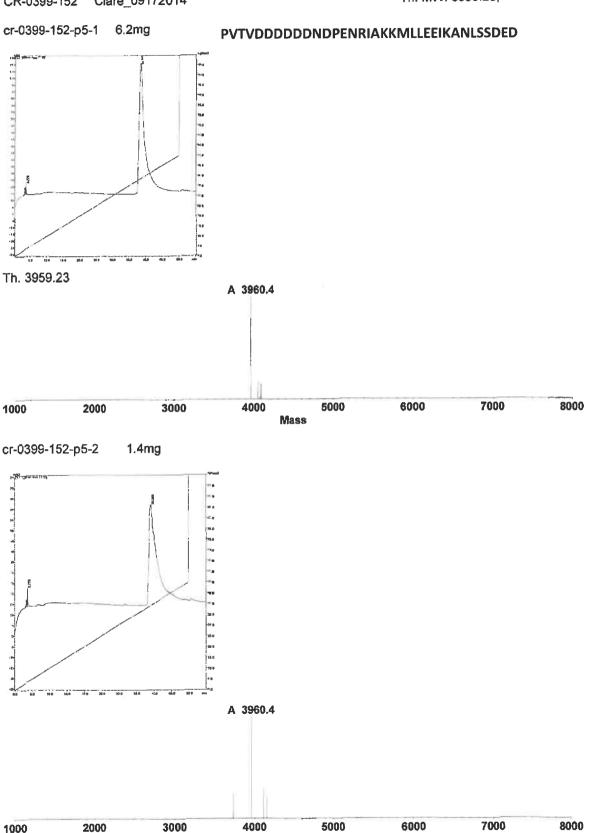
#### ATRX 1255-1289



1000

Th. MW. 3959.23,

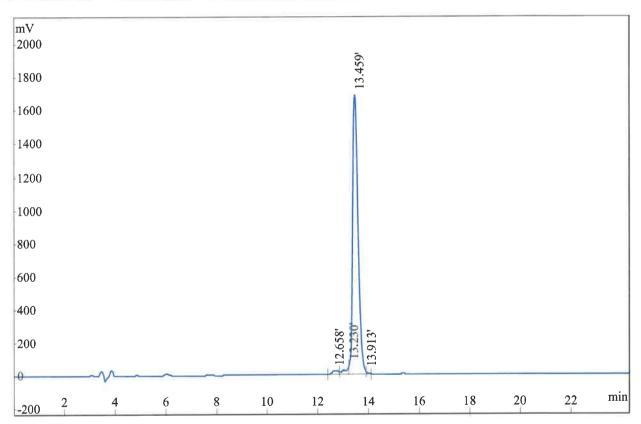


Mass

Sequence: DDDDNDPENRIAKKMLLEEIKANLSSDEDG	Name: ATRX6190

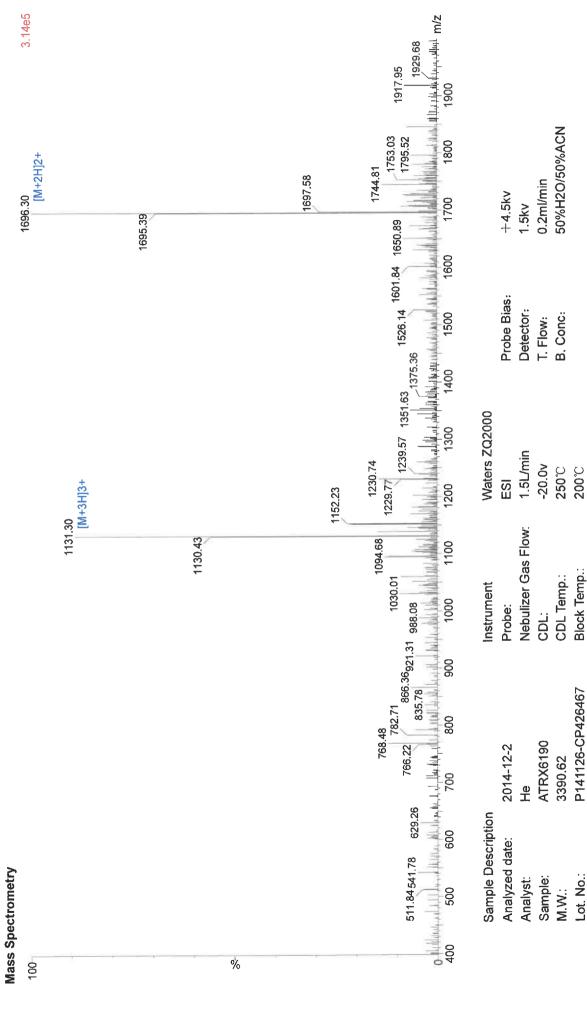
<b>M.W [M+H<sup>+</sup>]</b> : 3390.62	<b>Purity</b> : 96.31%	Amount: 9.5mg
101.00 [101.11 ]. 0000.02	· unity: 00.0170	

The second second			·	
HPLC Result				
Lot. No.:	P141126-CP	426467		
Column:	Gemini-NX	5μ C18 110A, 4	.6*250mm	
Solvent A	A: 0.1% Trit	luoroacetic Acid	l in 100% Acetonitrile	
Solvent B	B: 0.1% Trif	luoroacetic Acid	l in 100% Water	
Gradient:		A	В	
	0.0min	20%	80%	
	25.0min	45%	55%	
	25.1min	100%	0%	
	30.0min	Stop		
Volume: 10µl	Wavelength:	220nm Flo	w rate: 1.0ml/min	



Rank	Time	Conc.	Area
1	12. 658	1. 4265	342298
2	13.230	2.0804	499201
3	13.459	96.3156	23111416
4	13.913	0. 1775	42585
\ <u></u>			
Total		100.0000	23995500

## ATRX 1261-1290



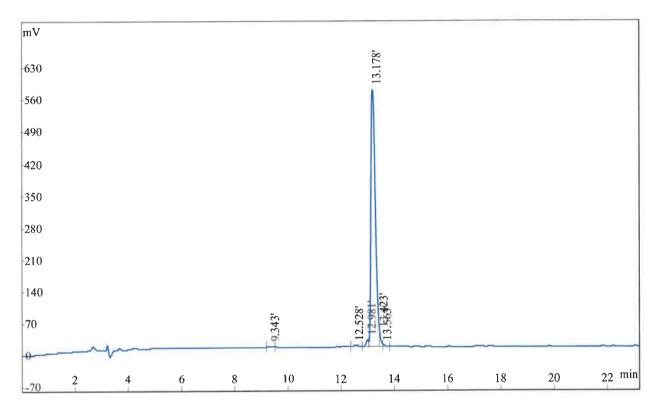
Page 3 of 3

#### ATRX 1263-1290

Sequence: DDNDPENRIAKKMLLEEIKANLSSDEDG Name: ATRX6390

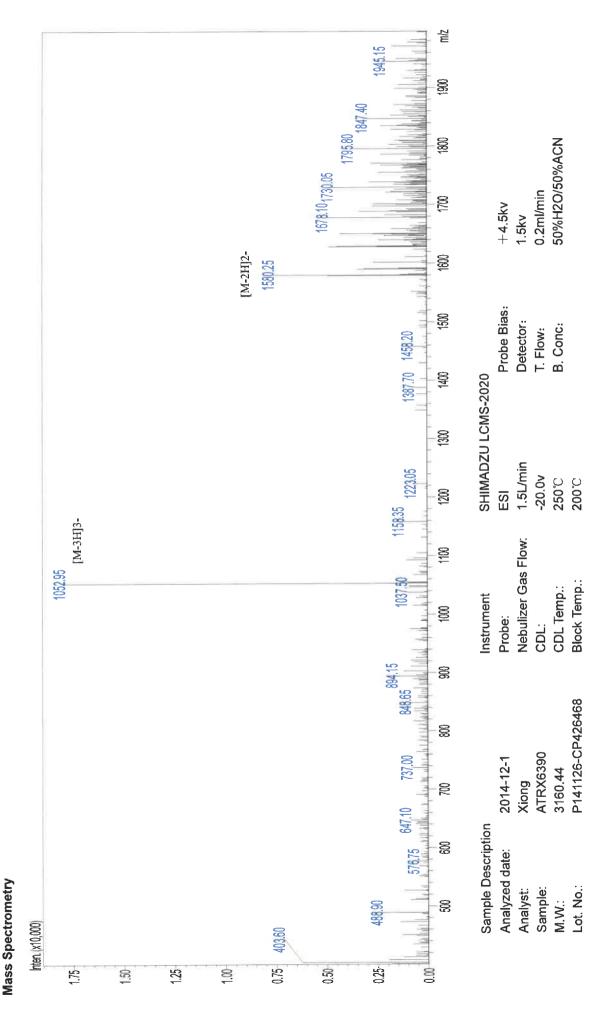
**M.W [M+H<sup>+</sup>]**: 3160.44 **Purity**: 95.44% **Amount**: 9.3mg

HPLC Result				
Lot. No.:	P141126-CP42	26468		
Column:	Gemini-NX 5	ı C18 110	A, 4.6*250mm	
Solvent A	A: 0.1% Triflu	oroacetic	Acid in 100% Acetonitrile	
Solvent B	B: 0.1% Triflu	oroacetic	Acid in 100% Water	
Gradient:		Α	В	
	0.0min	20%	80%	
	25.0min	45%	55%	
	25.1min	100%	0%	
	30.0min	Stop		
Volume: 10µl	Wavelength: 2	20nm	Flow rate: 1.0ml/min	



Rank	Time	Conc.	Area	
1	9.343	0.3266	19906	-2
2	12.528	0.7607	46361	
3	12.981	1.5852	96614	
4	13.178	95.4481	5817438	
5	13.423	1.5965	97302	
6	13.563	0.2829	17240	
Total		100.0000	6094861	

ATRX 1263-1290



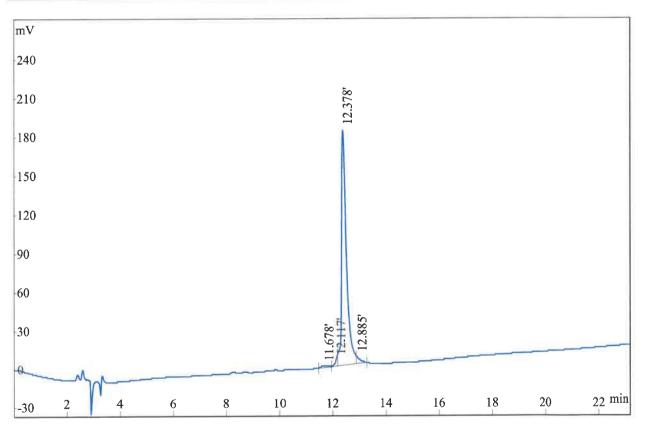
Page 3 of 3

#### ATRX 1265-1290

Sequence: NDPENRIAKKMLLEEIKANLSSDEDG Name: ATRX6590

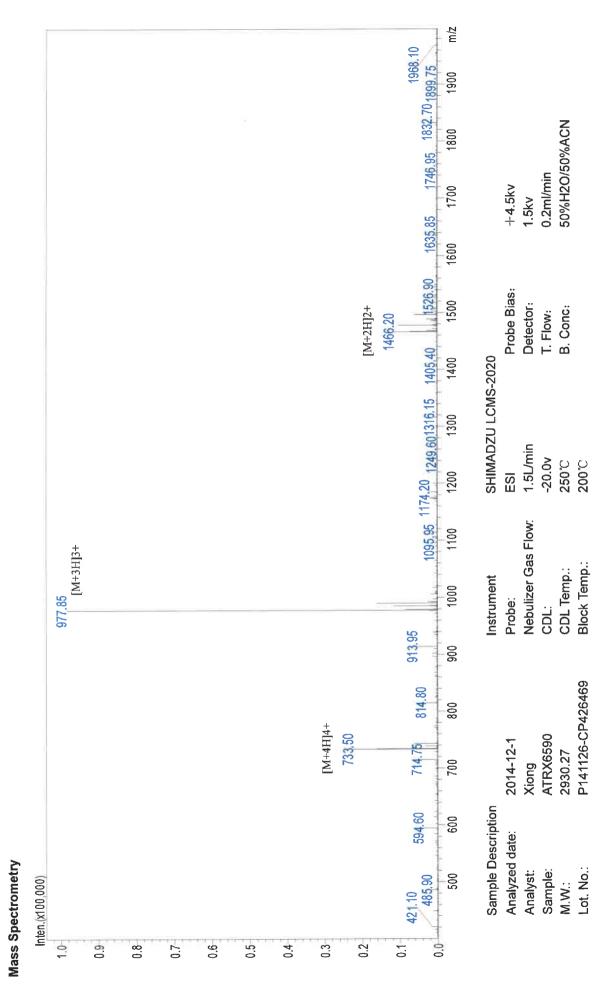
**M.W [M+H<sup>+</sup>]**: 2930.27 **Purity**: 95.13% **Amount**: 9.5mg

HPLC Result				
Lot. No.:	P141126-CP4	426469		
Column:	Gemini-NX	Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	A: 0.1% Trifl	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile		
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water			
Gradient:		Α	В	
	0.0min	20%	80%	
	25.0min	45%	55%	
	25.1min	100%	0%	
	30.0min	Stop		
Volume: 10µl	Wavelength:	220nm	Flow rate: 1.0ml/min	



Rank	Time	Conc.	Area
1	11.678	0.9523	21572
2	12.117	1.7914	40581
3	12.378	95.1301	2154930
4	12.885	2.1262	48163
Total		100.0000	2265246

ATRX 1265-1290

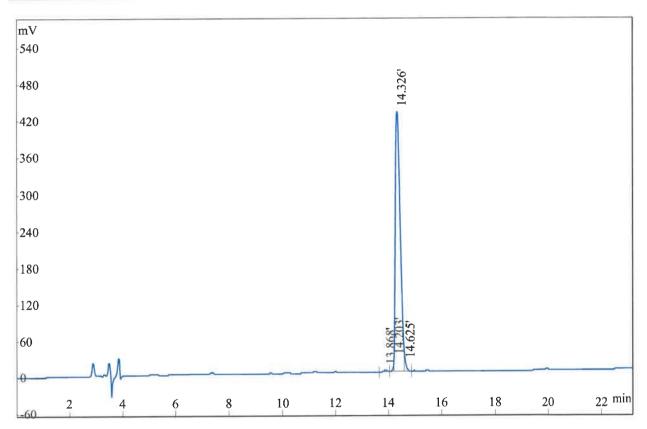


Page 3 of 3

Sequence: PENRIAKKMLLEEIKANLSSDEDG Name: ATRX6790

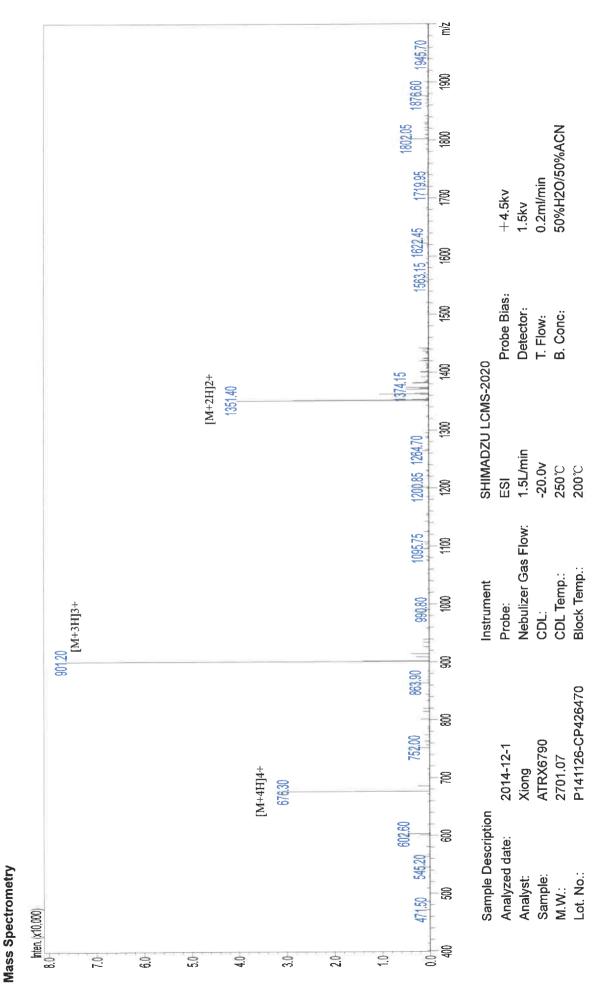
M.W [M+H<sup>+</sup>]: 2701.07 Purity: 97.35% Amount: 9.6mg

HPLC Result				
Lot. No.:	P141126-CP	426470		
Column:	Gemini-NX	5μ C18 110A, 4	6*250mm	
Solvent A	A: 0.1% Trif	luoroacetic Acid	in 100% Acetonitrile	
Solvent B	B: 0.1% Trif	luoroacetic Acid	in 100% Water	
Gradient:		Α	В	
	0.0min	20%	80%	
	25.0min	45%	55%	
	25.1min	100%	0%	
	30.0min	Stop		
Volume: 10µl	Wavelength:	220nm Flo	w rate: 1.0ml/min	



Rank	Time	Conc.	Area
1	13.868	0.3909	20035
2	14.203	0.6142	31478
3	14.326	97.3510	4988946
4	14.625	1.6439	84247
Total		100.0000	5124706

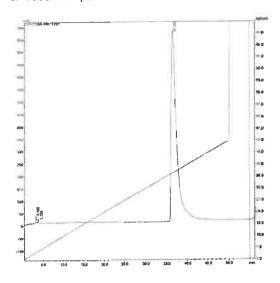
ATRX 1267-1290



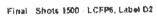
Page 3 of 3

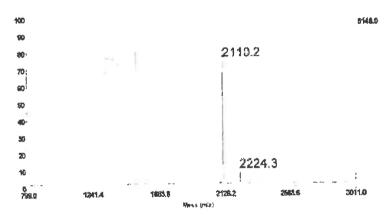
Clare-10102014, PENRIAKKMLLEEIKANL-NH2

cr-0399-124-p2-1



Th. MH+: 2010.57







11 Duerdin Street Clayton Victoria 3168 Australia Tel +61 3 9565 1111 Fax +61 3 9565 1199 mimotopes@mimotopes.com www.mimotopes.com

## Pertificate of Analysis

ATRX 1264-1285

#### **Custom Synthesized Peptide**

Order Number:

27797

Peptide Number:

2779701

Theoretical Molecular Weight:

2.541.90

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

H-DNDPENRIAKKMLLEEIKANLS-OH

At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid

Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.

Requested:	5mg at	90% by HPLC		
Supplied :				
Tube	Mass (mg)	Minimum Purity (%)		
1	9.5	93		

Comments : Amino Acid Code Sequence : Asp-Asn-Asp-Pro-Glu-Asn-Arg-lie-Ala-Lys-Lys-Met-Leu-Leu-Glu-Glu-IIe-Lys-Ala-Asn-Leu-Ser Monday, 24 October 2016 Quality Assured by Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotopes.com For Material Safety Data information please email: mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS

CONC DAXX = 97.5 MM.



**Product Name** 

2779701

Lot No

P2779701-JQ537508

Column

4.6×250mm, YMC-Triart C18

Solvent A

0.1% trifluoroacetic in 100% acetonitrile

Solvent B

0.1% trifluoroacetic in 100% water

Gradient

Α В

0.01min 25min

10% 90% 67%

33% 0%

25.1min 100% 30min

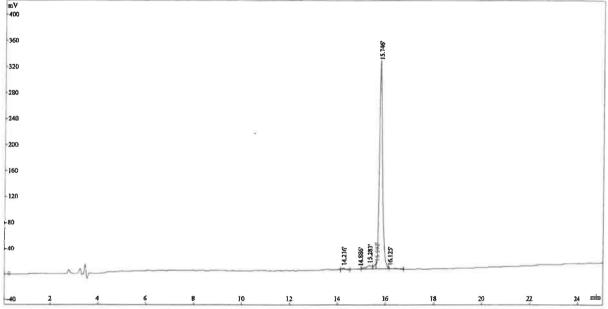
**STOP** 

Flow rate Wavelength 1.0 mL/min

214nm

Volume

5ul



Rank	Time	Conc.	Area	Height	
1	14.216	0.6066	19280	2667	
2	14.886	0.7783	24736	1795	
3	15.283	3.483	110704	5601	
4	15.592	1.688	53637	8454	
5	15.746	92.53	2940776	326979	
6	16.125	0.9064	28807	1831	
Total		100	13762734	1361070	



### Pertificate of Analysis

SPEP 1

#### **Custom Synthesized Peptide**

Order Number :

29315

Peptide Number

2931501

Theoretical Molecular Weight:

2.738.12

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

H-SDDNDPEN(1151)IAK(1151)MLLEEIKANLS-OH

At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid.

Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.

(1151) in sequence means (S)-2-(4'-pentenyl) alanine

Requested:

1mg at 90% by HPLC

Supplied:

Tube Mass (mg) Minimum

Solubility Information Soluble

Purity (%) Solubility mg/mL

0-20% Acetonitrile in 100Mm Ammonium Bicarbonate

6.0

Comments : Amino Acid Code Sequence :

Ser-Asp-Asp-Asn-Asp-Pro-Glu-Asn-PntAla-Ile-Ala-Lys-PntAla-Met-Leu-Leu-Glu-Glu-Ile-Lys-Ala-Asn-

Supplied as stapled peptide.

LCMS analysis the molecular ions consistent with the parent are found throughout the peak at 7,14 mins.

The reported purity is the sum under the peaks 11.52-12.32 observed in the HPLC trace.

Quality Assured by:

Monday, 30 April 2018

. Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotopes.com For Material Safety Data information please email: mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS

<sup>\*</sup> Solubility Information provided was as used in the purification processes and is for information only. Many other solubilization conditions may be effective and we advise trying simpler methods first. (See 'A Guide to Handling and Storing Peptides').



## Pertificate of Analysis

SPEP 2

#### **Custom Synthesized Peptide**

Order Number:

29315

Peptide Number:

2931502

Theoretical Molecular Weight:

2.778.12

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

H-SDDNDPENR(1151)AKK(1151)LLEEIKANLS-OH

At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid.

Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left. (1151) in sequence means (S)-2-(4'-pentenyl) alanine

Requested:

1mg at 90% by HPLC

Supplied:					
Tube	Mass (mg)	Minimum Purity (%)	Solubility Information Solubility	mg/mL	Buffer
1	6.1	93	Soluble	6.0	0-20% Acetonitrile in 100Mm Ammonium Bicarbonate
<b>-</b> 2	2.2	98	Soluble	6.0	0-20% Acetonitrile in 100Mm Ammonium Bicarbonate

Solubility information provided was as used in the purification processes and is for information only. Many other solubilization conditions may be effective and we advise trying simpler methods first. (See 'A Guide to Handling and Storing Peptides').

Comments : Amino Acid Code Sequence :

Ser-Asp-Asp-Asn-Asp-Pro-Glu-Asn-Arg-PntAla-Ala-Lys-Lys-PntAla-Leu-Leu-Glu-Glu-IIe-Lys-Ala-Asn-

Leu-Ser

Supplied as stapled peptide.

LCMS analysis of tube 2 indicates the molecular ions consistent with the parent are found throughout the peak at 6.34mins.

The reported purity is the sum under the two peaks observed in the HPLC trace.

Quality Assured by :

Friday, 27 April 2018

Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotoFor Material Salety Data information please email: mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS peptide\_support\_group@mimotopes.com



## Certificate of Analysis

SPEP 3

#### **Custom Synthesized Peptide**

Order Number: 29315  Peptide Number: 2931503  Theoretical Molecular Weight: 2,765.18  Observed Molecular Weight: MS Analysis detected molecular ions consistent with the parent  Sequence: H-SDDNDPENRIAK(1151)MLL(1151)EIKANLS-OH  At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid  Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.						
Theoretical Molecular Weight: 2,765.18  Observed Molecular Weight: MS Analysis detected molecular ions consistent with the parent  Sequence: H-SDDNDPENRIAK(1151)MLL(1151)EIKANLS-OH  At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid  Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.						
Observed Molecular Weight: MS Analysis detected molecular ions consistent with the parent  Sequence: H-SDDNDPENRIAK(1151)MLL(1151)EIKANLS-OH  At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid  Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.						
Sequence: H-SDDNDPENRIAK(1151)MLL(1151)EIKANLS-OH  At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.						
At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.						
Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.						
(1151) in sequence means (S)-2-(4'-pentenyl) alarine  Requested: 1mg at 90% by HPLC						
Supplied:						
Tube Mass (mg) Minimum Solubility Information Purity (%) Solubility mg/mL Buffer 1 4.3 95 Soluble 7.5 0-20% Acetonitrile in 100Mm Ammonium Bicarbonate						
* Solubility information provided was as used in the purification processes and is for information only. Many other solubilization conditions may be effective and we advise trying simpler methods first. (See 'A Guide to Handling and Storing Peptides').  Comments: Amino Acid Code Sequence: Ser-Asp-Asp-Asn-Asp-Pro-Glu-Asn-Arg-Ile-Ala-Lys-PntAla-Met-Leu-Leu-PntAla-Glu-Ile-Lys-Ala-Asn-Leu-Ser Supply as stapled peptide.  Quality Assured by:  Tuesday, 24 April 2018  C. Pham - Quality Assurance Department  For any technical enquiries please contact: peptide_support_group@mimotopes.com For Material Safety Data information please email: mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS						



## Pertificate of Analysis

SPEP 4

#### **Custom Synthesized Peptide**

Order Num	ber !!!		29315					
•			2931504					
Peptide Nu			_,,,,,					
Theoretical	Molecular We	eight :	2,822.28					
Observed N	vlolecular Wei	ght:	MS Analysis	detected	molecular ions consistent with the parent			
Sequence :	Sequence: H-SDDNDPENRIAR		KMLL(1151)EIK(	MLL(1151)EIK(1151)NLS-OH				
At the N-terminus, Sequence is in standard s (1151) in sequence mean			ingle letter code (unles:	<ul> <li>means Free amine. At the C-terminus, -OH means Free acid.</li> <li>gle letter code (unless otherwise specified), amino terminus is on the left.</li> <li>(S)-2-(4'-pentenyl) alanine</li> </ul>				
Requested	: 1mg at	90% by HPL	.c					
Supplied :								
Tube	Mass (mg)	Minimum Purity (%) 94	Solubility Information Solubility Soluble	mg/mL	Buffer 100% H2O			
Comments Amino Acid Ser-Asp-As Leu-Ser	ve advise trying si : I Code Seque	mpler methods t nce : ro-Glu-Asn-A	first. (See 'A Guide to	Handung ar	information only. Many other solubilization conditions made Storing Peptides').  J-Leu-PntAla-Glu-IIe-Lys-PntAla-Asn-	ay be		
Quality Assured by :				Friday, 27 April 2018				
	1	Pham - Q	uality Assurance I	Departme	ent			
For Materia	hnical enquiri al Safety Data @mimotopes	information	ntact: peptide please email : sting "Custom Syr		t_group@mimotopes.com Peptide" MSDS			



## Pertificate of Analysis

SPEP 5

#### **Custom Synthesized Peptide**

Order Number:

29315

Peptide Number:

2931505

Theoretical Molecular Weight:

2.822.28

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

H-SDDNDPENR(1152)AKKMLL(1151)EIKANLS-OH

At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid. Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left. (1151) in sequence means (S)-2-(4'-pentenyl) alanine; (1152) in sequence means (R)-2-(7'-octenyl) alanine

Requested:

1mg at 90% by HPLC

Supplied:

. 1

Tube

Mass (mg)

Minimum

Purity (%)

Soluble

Solubility Information Solubility

mg/mL 6.0

0-20% Acetonitrile in 100Mm Ammonium Bicarbonate

Amino Acid Code Sequence:

Ser-Asp-Asp-Asn-Asp-Pro-Glu-Asn-Arg-OctAla-Ala-Lys-Lys-Met-Leu-Leu-PntAla-Glu-Ile-Lys-Ala-Asn-

Leu-Ser

Supplied as stapled peptide.

LCMS analysis indicates molecular ions consistent with the parent are found throughout the peak at 6.8 mins.

The reported purity is the sum under the two peaks observed in the HPLC trace.

Quality Assured by:

Monday, 30 April 2018

Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotopes.com For Material Safety Data information please email: mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS

<sup>\*</sup> Solubility information provided was as used in the purification processes and is for information only. Many other solubilization conditions may be effective and we advise trying simpler methods first. (See 'A Guide to Handling and Storing Peptides').



## Pertificate of Analysis

SPEP 6

#### **Custom Synthesized Peptide**

Order Number:

29315

Peptide Number:

2931506

Theoretical Molecular Weight:

2.808.21

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

H-SDDNDPENRIAK(1152)MLLEEI(1151)ANLS-OH

At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid. Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left. (1151) in sequence means (S)-2-(4'-pentenyl) alanine; (1152) in sequence means (R)-2-(7'-octenyl) alanine

Requested:

1mg at 90% by HPLC

	Supplied :					
	Tube	Mass (mg)	Minimum Purity (%)	Solubility Information Solubility	mg/mL	Buffer
	1	3.5	90`	Soluble	6.0	0-20% Acetonitrile in 100Mm Ammonium Bicarbonate
41	2	3.7	91	Soluble	6,0	0-20% Acetonitrile in 100Mm Ammonium Bicarbonate

<sup>\*</sup> Solubility information provided was as used in the purification processes and is for information only. Many other solubilization conditions may be effective and we advise trying simpler methods first. (See 'A Guide to Handling and Storing Peptides').

Comments: Amino Acid Code Sequence:

Ser-Asp-Asp-Asn-Asp-Pro-Glu-Asn-Arg-Ile-Ala-Lys-OctAla-Met-Leu-Leu-Glu-Glu-Ile-PntAla-Ala-Asn-

Supplied as stapled peptide.

LCMS analysis indicates molecular ions consistent with the parent are found throughout the peak at 7.5 and 7.8mins.

The reported purity is the sum under the peaks between 11.86 and 12.73 min the HPLC trace.

Quality Assured by :

Friday, 27 April 2018

Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotopes.com For Material Salety Data information please email: mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS



## Pertificate of Analysis

SPEP 7

#### **Custom Synthesized Peptide**

Order Number:

29315

Peptide Number:

2931507

Theoretical Molecular Weight:

2,862,28

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

H-SDDNDPENRIAKK(1152)LLEEIK(1151)NLS-OH

At the N-terminus, H- means Free amine. At the C-terminus, -OH means Free acid. Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left. (1151) in sequence means (S)-2-(4'-pentenyl) alanine; (1152) in sequence means (R)-2-(7'-octenyl) alanine

6.0

Requested:

1mg at 90% by HPLC

Supplied .

Tube Mass (mg) Minimum Purity (%) Solubility Information Solubility

Soluble

0-20% Acetonitrile in 100Mm Ammonlum Bicarbonate

Comments : Amino Acid Code Sequence :

Ser-Asp-Asp-Asn-Asp-Pro-Giu-Asn-Arg-lie-Ala-Lys-Lys-OctAla-Leu-Leu-Giu-Giu-Ile-Lys-PntAla-Asn-

Supplied as stapled peptide.

LCMS analysis indicates molecular ions consistent with the parent are found throughout the peak at 6.8mins.

The reported purity is the sum under the two peaks observed in the HPLC trace.

Quality Assured by:

Friday, 27 April 2018

C. Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotopes.com For Material Safety Data information please email : mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS

<sup>\*</sup> Solubility information provided was as used in the purification processes and is for information only. Many other solubilization conditions may be effective and we advise trying simpler methods first. (See 'A Guide to Handling and Storing Peptides').



#### CERTIFICATE OF ANALYSIS

**Product Name** 

Pep1

Lot No

JT-79065

Sequence

FAM-DNDPENRIAKK[(S)-2-(4'-pentenyl)

alanine]LLEEIK[(R)-2-7-(7'-octenyl)alanine]NLS-NH2

Dissolution condition

15%ACN+85%H2O

Length

AA

Modification

N/A

Molecular Weight (MW)

2989.38

Storage

-20℃

Test Items	Specifications	Results
Purity by HPLC	90%	93.50%
Peptide Content	N/A	N/A
Moisture content	N/A	N/A
Acetic acid content	N/A	N/A
Appearance	yellow lyophilized powder	Conforms
Quantity	5mg	5.0mg

Certified by:

**Quality Assurance** 

Department

Date 01/16/2019

Note: this product is intended for research use only; not for diagnostic or human use.



#### Sample Information

Order ID : Syn-79065

Name : Pep1

Sequence : FAM-DNDPENRIAKK[(S)-2-(4'-pentenyl)

alanine]LLEEIK[(R)-2-7-(7'-octenyl)alanine]NLS-NH2

Lot No : JT-79065

Pump A : 0.1% Trifluoroacetic in 100% Water

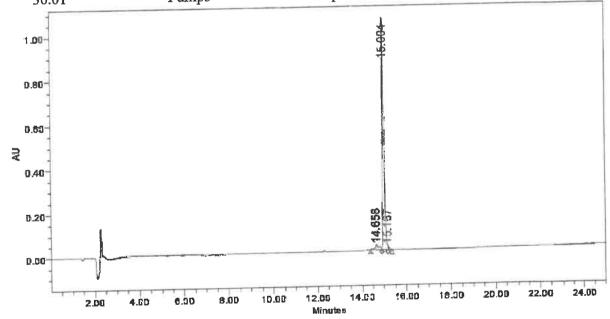
Pump B : 0.1% Trifluoroacetic in 100% Acetonrtrile

Total Flow : 1ml/min
Wavelength : 220nm

Analytial column type : SHIMADZU Inertsil ODS-SP (4.6\*250mm\*5um)

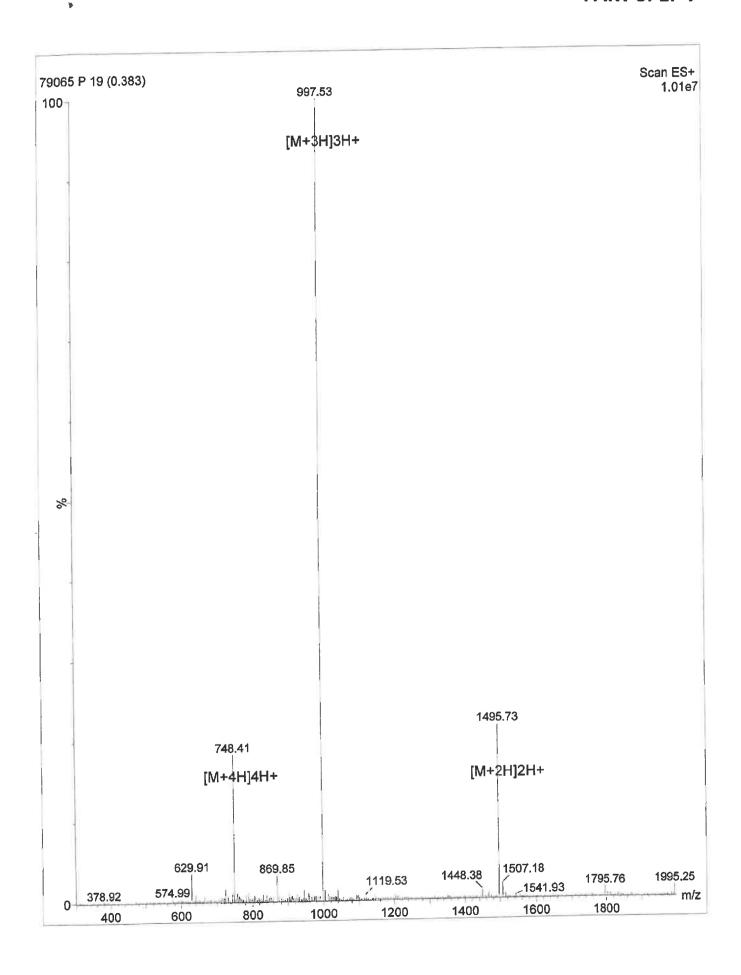
Inj. Volume : 30ul

mj. Volumo	•		77.1
Time	Module	Action	Value
0.00	Pumps	B.Conc	10
25.00	Pumps	B.Conc	70
25.01	Pumps	B.Conc	100
	•	B.Cone	100
30.00	Pumps		100
30.01	Pumps	Stop	

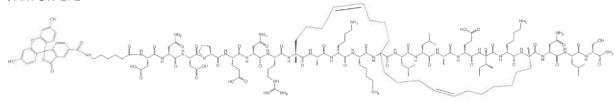


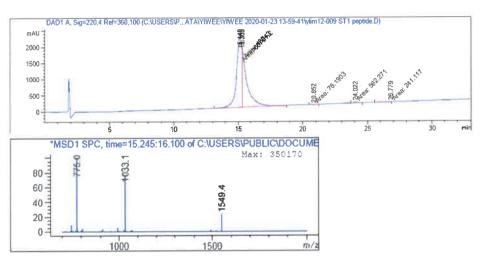
	RT	Area	% Area	Height
1	14.658	281938	5.30	23866
2	15.004	4974285	93.50	1034265
3	15.167	64027	1.20	17979

#### Synpeptide Co., Ltd



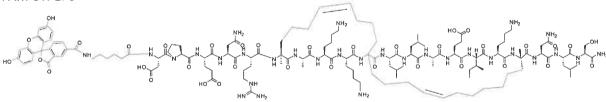
#### FAM-STPEP2

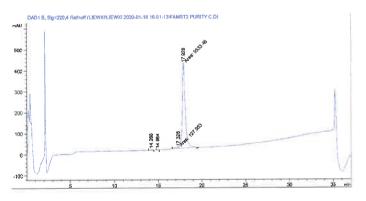


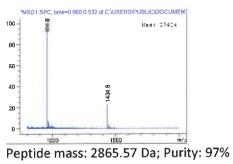


Peptide mass: 3094.64 Da; Purity: 99%

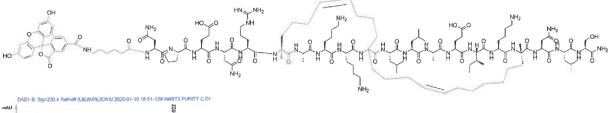
#### FAM-STPEP3

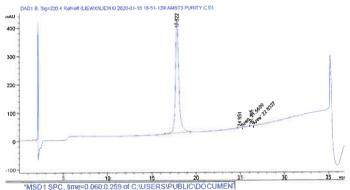


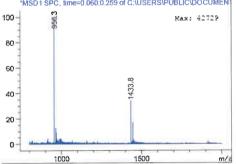




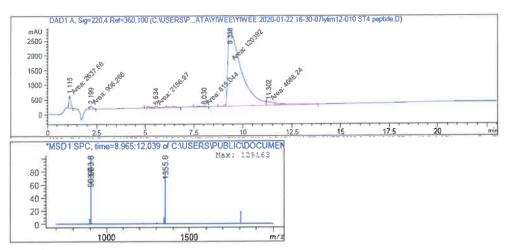
#### FAM\_STPEP4







Peptide mass: 2864.58 Da; Purity: 99%



Peptide mass: 2707.45 Da; Purity: 91%

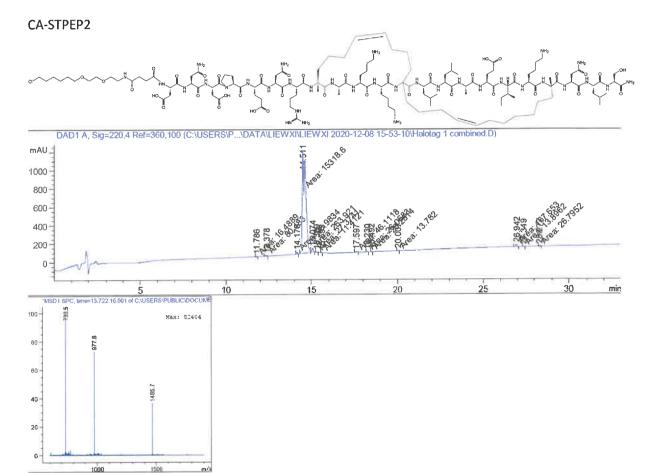
General information: RAMAGE resin was obtained from PCAS Biomatrix. Fmoc-amino acids, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium (HATU), and 1-Hydroxy-7-azabenzotriazole (HOAt) were obtained from Advanced Chemtech (Louisville, KY). 5-carboxy fluorescein was purchased from Beijing Okeanos Tech (Beijing, China). Trifluoroacetic acid (TFA) and N, N-Diisopropylethylamine (DIPEA) was purchased from Tokyo Chemical Industry (Tokyo, Japan) while all other solvents and reagents were obtained from Fisher Scientific (Loughborough, United Kingdom). All reagents were used as received.

Solid-phase peptide synthesis: The linear peptide was synthesized on the CEM peptide synthesizer. 1 M OxymaPure with 0.1 M DIPEA in DMF was used as the coupling agent and 0.5 M DIC in DMF was the activating agent. Fmoc deprotection was done with 20% Piperidine in DMF. Following ring closing metathesis, 5-carboxy fluorescein was coupled onto the N-terminus manually with pre-activated solutions (7 min) of 2.8 eq. HATU, 3 eq. HOAt, and 6 eq. DIPEA for 1 h.

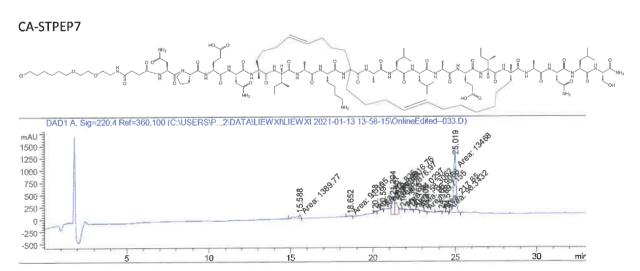
Ring closing metathesis: 0.2 equiv. Grubbs I catalyst was dissolved in DCE (5 mg/mL) was bubbled in the resin for four times for two hours each.

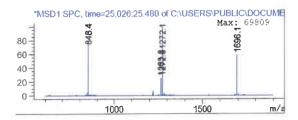
Cleavage: Following synthesis, the peptide resin was shrunk and dried with washes of methanol and diethyl ether. It was cleaved from the resin with a cleavage cocktail of TFA/Tips/H2O (95:2.5:2.5) for 2 h. The resin was filtered off and peptide precipitated in diethyl ether (50 mL). The peptide was re-dissolved in a mixture of ACN/H2O (1:1) and lyophilised.

Peptide purification and analysis: The dried peptide was re-dissolved in acetonitrile and water (1:1) and purified via reverse-phase HPLC using an Agilent 1260 Infinity system fitted with a Phenomenex preparative column (Jupiter C12, 4 μm, Proteo 90 Å, 250 x 10 mm). Eluents used were 0.1% aqueous TFA in water and 0.1% TFA in acetonitrile. Peptide purity and molecular weight were confirmed via UPLC-MS using an Agilent 1260 Infinity II system fitted with a Phenomenex analytical column (Aeris 1.7 μm, Peptide XB-C18 100 LC Column, 150 x 2.1 mm). Eluents used were 0.1% aqueous formic acid in water and 0.1% formic acid in acetonitrile.



Peptide mass: 2930.91 Da; Purity: 97%





Peptide mass: 2543.52 Da; Purity: 60%

General information: RAMAGE resin was obtained from PCAS Biomatrix. Fmoc-amino acids, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium (HATU), and 1-Hydroxy-7-azabenzotriazole (HOAt) were obtained from Advanced Chemtech (Louisville, KY). Halotag was purchased from Beijing Okeanos Tech (Beijing, China). Trifluoroacetic acid (TFA) and N, N-Diisopropylethylamine (DIPEA) was purchased from Tokyo Chemical Industry (Tokyo, Japan) while all other solvents and reagents were obtained from Fisher Scientific (Loughborough, United Kingdom). All reagents were used as received.

Solid-phase peptide synthesis: The linear peptide was synthesized on the CEM peptide synthesizer. 1 M OxymaPure with 0.1 M DIPEA in DMF was used as the coupling agent and 0.5 M DIC in DMF was the activating agent. Fmoc deprotection was done with 20% Piperidine in DMF. Following ring closing metathesis, Halotag was coupled onto the N-terminus manually with pre-activated solutions (7 min) of 2.8 eq. HATU, 3 eq. HOAt, and 6 eq. DIPEA for 1 h.

Ring closing metathesis: 0.2 equiv. Grubbs I catalyst was dissolved in DCE (5 mg/mL) was bubbled in the resin for four times for two hours each.

Cleavage: Following synthesis, the peptide resin was shrunk and dried with washes of methanol and diethyl ether. It was cleaved from the resin with a cleavage cocktail of TFA/Tips/H2O (95:2.5:2.5) for 2 h. The resin was filtered off and peptide precipitated in diethyl ether (50 mL). The peptide was re-dissolved in a mixture of ACN/H2O (1:1) and lyophilised.

Peptide purification and analysis: The dried peptide was re-dissolved in acetonitrile and water (1:1) and purified via reverse-phase HPLC using an Agilent 1260 Infinity system fitted with a Phenomenex preparative column (Jupiter C12, 4 μm, Proteo 90 Å, 250 x 10 mm). Eluents used were 0.1% aqueous TFA in water and 0.1% TFA in acetonitrile. Peptide purity and molecular weight were confirmed via UPLC-MS using an Agilent 1260 Infinity II system fitted with a Phenomenex analytical column (Aeris 1.7 μm, Peptide XB-C18 100 LC Column, 150 x 2.1 mm). Eluents used were 0.1% aqueous formic acid in water and 0.1% formic acid in acetonitrile.



## Pertificate of Analysis

#### **Custom Synthesized Peptide**

Order Number	
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32113

Peptide Number ::

3211301

Theoretical Molecular Weight:

2,312.52

Observed Molecular Weight:

MS Analysis detected molecular ions consistent with the parent

Sequence:

Ac-AMDDLMLSPDDIEQWFTED-NH2

At the N-terminus, Ac- means acetyl. At the C-terminus, -NH2 means Amide. Sequence is in standard single letter code (unless otherwise specified), amino terminus is on the left.

Requested:

5mg at 90% by HPLC

Supplied:

Tube

Mass (mg)

Minimum Purity (%)

Comments : Amino Acid Code Sequence :

Ala-Met-Asp-Asp-Leu-Met-Leu-Ser-Pro-Asp-Asp-Ile-Glu-Gln-Trp-Phe-Thr-Glu-Asp

Quality Assured by:

\_\_\_\_\_ Thursday, 25 June 2020

C. Pham - Quality Assurance Department

For any technical enquiries please contact: peptide\_support\_group@mimotopes.com
For Material Safety Data information please email:
mimotopes@mimotopes.com, requesting "Custom Synthesized Peptide" MSDS



Product Name 3211301

Lot No P3211301-HS807598

Column
Solvent A
Solvent B
Gemini-NX 5μ C18 110A, 4.6\*250mm
0.1%Trifluoroacetic in 100% Acetonitrile
0.1%Trifluoroacetic in 100% Water

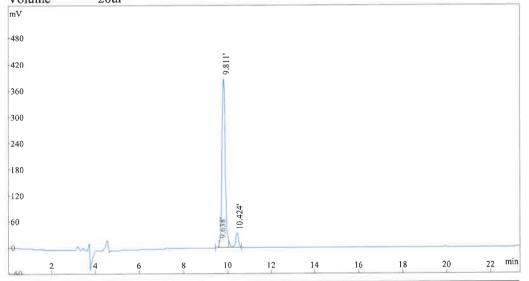
Gradient

A B

0.01min 30% 70% 25min 70% 30%

> 25.01min 100% 0% 30min Stop

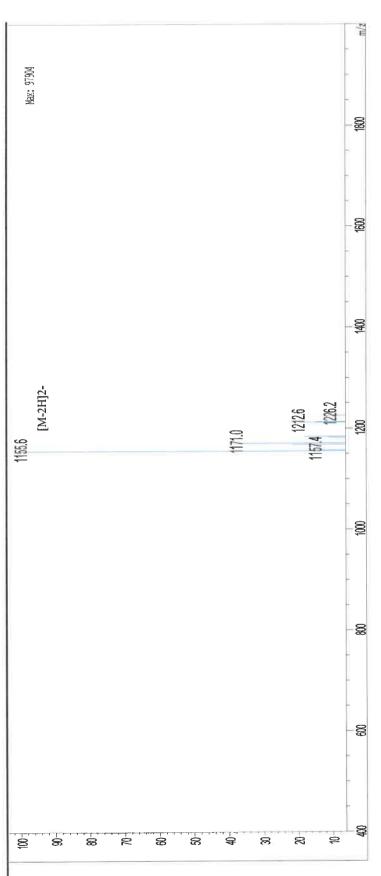
Flow rate 1.0ml/min Wavelength 214nm Volume 20ul



Rank	Time	Conc.	Area	Height
1	9. 638	0.7101	28115	14335
2	9. 811	90. 4273	3580304	389022
3	10. 424	8. 8626	350898	33662
Total		100	3959317	437019

# ANALYTICAL DATA





Data Acquired : 12/06/2020 14:16:21 PM

: 3211301 : 2312.48 Injection Volume: 1 Sample Name : 321 Mw

Lot No.

: P3211301-HS807598

Probe bias :+4.5kv Probe

Nebulizer Gas Flow :1.5L/min Detector :2.0kv
CDL :-20.0v T.Flow :0.2ml/min
CDL Temp :250°C B.conc :50%H2O/50%ACN
Block Temp :200°C CDL CDL Temp Block Temp