

# Phosphonodiamidate Prodrugs of Phosphoantigens (ProPAgens) Exhibit Potent V $\gamma$ 9/V $\delta$ 2 T Cell Activation and Eradication of Cancer Cells

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Figure S1. HPLC chromatograms of final ProPAgens.

# Analysis Report

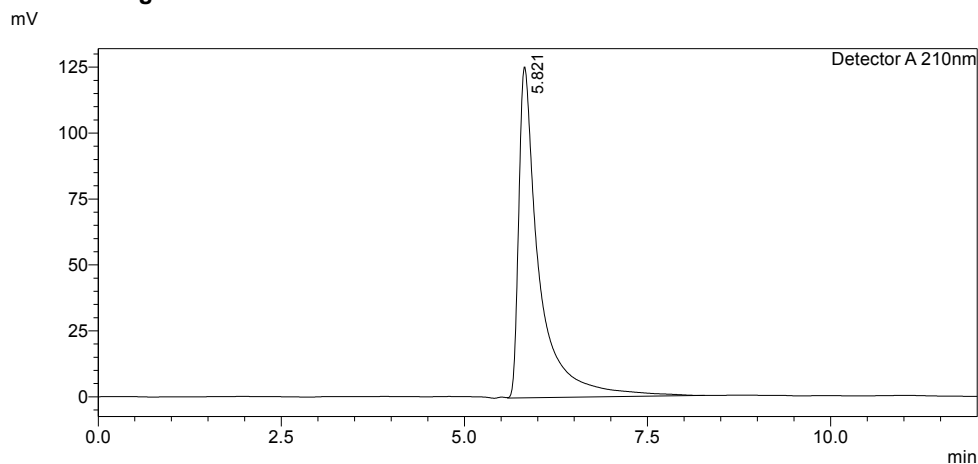
## <Sample Information>

Sample Name : DAFN-Me-1  
Sample ID : Me01  
Data Filename : XQ-DAFN-Me-1.lcd  
Method Filename : method diamidate.lcm  
Batch Filename : Batch Meisotbu01 310119.lcb  
Vial # : 1-1  
Injection Volume : 10 uL  
Date Acquired : 31/01/2019 14:55:21  
Date Processed : 31/01/2019 15:07:21

**ProPAgen 9a**

Sample Type : Unknown  
Acquired by : System Administrator  
Processed by : System Administrator

## <Chromatogram>



## <Peak Table>

Detector A 210nm				
Peak#	Ret. Time	Area	Height	Area%
1	5.821	2444504	125392	100.000
Total		2444504	125392	100.000

# Analysis Report

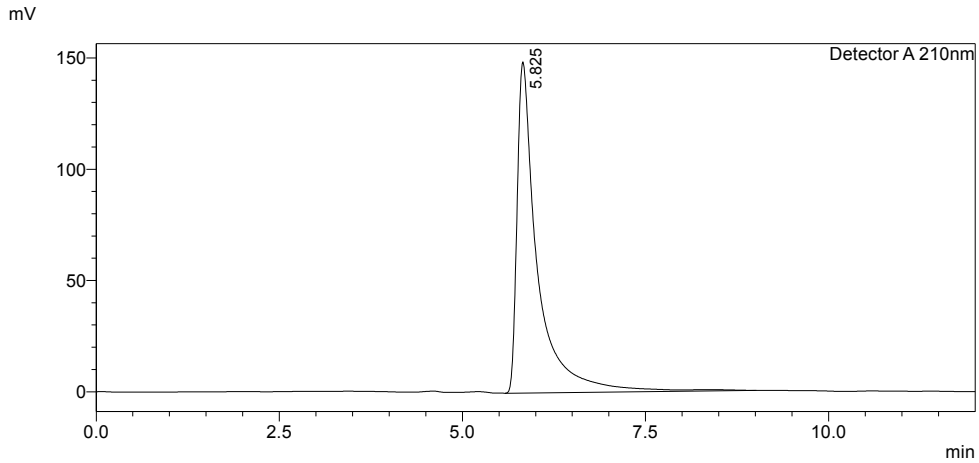
**<Sample Information>**

Sample Name : DAFN-Iso-1  
 Sample ID : Iso01  
 Data Filename : XQ-DAFN-Iso-1.lcd  
 Method Filename : method diamidate.lcm  
 Batch Filename : Batch Meisotbu01 310119.lcb  
 Vial # : 1-2  
 Injection Volume : 10 uL  
 Date Acquired : 31/01/2019 15:07:40  
 Date Processed : 31/01/2019 15:19:41

**ProPagen 9b**

Sample Type : Unknown  
 Acquired by : System Administrator  
 Processed by : System Administrator

**<Chromatogram>**



**<Peak Table>**

Detector A 210nm				
Peak#	Ret. Time	Area	Height	Area%
1	5.825	2891023	148697	100.000
Total		2891023	148697	100.000

# Analysis Report

## <Sample Information>

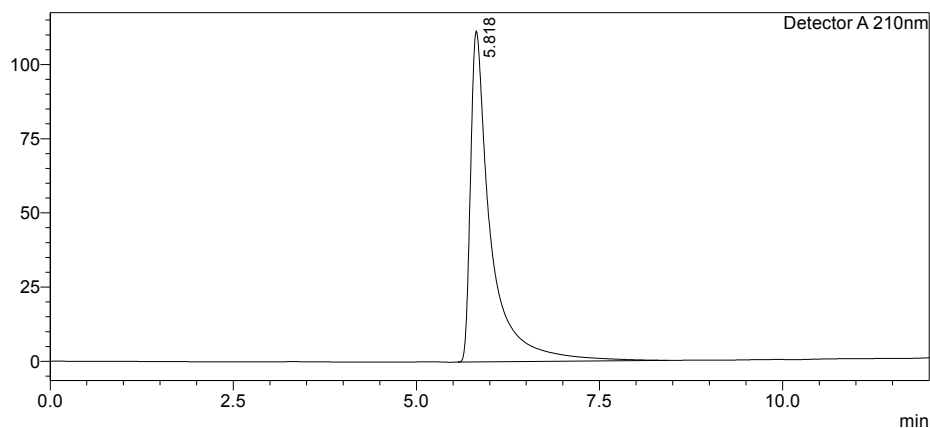
Sample Name : tBu-004  
 Sample ID : tBu004  
 Data Filename : XQ-DAFN-tBU-004.lcd  
 Method Filename : method diamidate.lcm  
 Batch Filename : batch05 diamidate 040219.lcb  
 Vial # : 1-1  
 Injection Volume : 5 uL  
 Date Acquired : 04/02/2019 15:35:14  
 Date Processed : 04/02/2019 15:47:14

**ProPagen 9c**

Sample Type : Unknown  
 Acquired by : System Administrator  
 Processed by : System Administrator

## <Chromatogram>

mV



## <Peak Table>

Detector A 210nm

Peak#	Ret. Time	Area	Height	Area%
1	5.818	2145865	111485	100.000
Total		2145865	111485	100.000

# Analysis Report

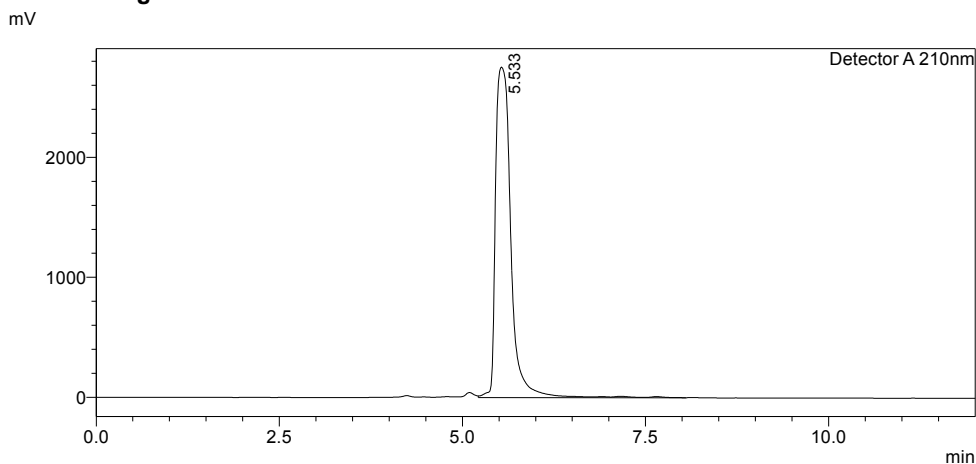
## <Sample Information>

Sample Name : DAFN-BN-001  
 Sample ID : BN-001  
 Data Filename : XQ-DAFN-Bn-2.lcd  
 Method Filename : method diamidate.lcm  
 Batch Filename : Batch 0.7mlmin 310119.lcb  
 Vial # : 1-1  
 Injection Volume : 10 uL  
 Date Acquired : 31/01/2019 14:16:14  
 Date Processed : 31/01/2019 14:28:15

**ProPagen 9d**

Sample Type : Unknown  
 Acquired by : System Administrator  
 Processed by : System Administrator

## <Chromatogram>



## <Peak Table>

Detector A 210nm				
Peak#	Ret. Time	Area	Height	Area%
1	5.533	40083588	2753823	100.000
Total		40083588	2753823	100.000

# Analysis Report

## <Sample Information>

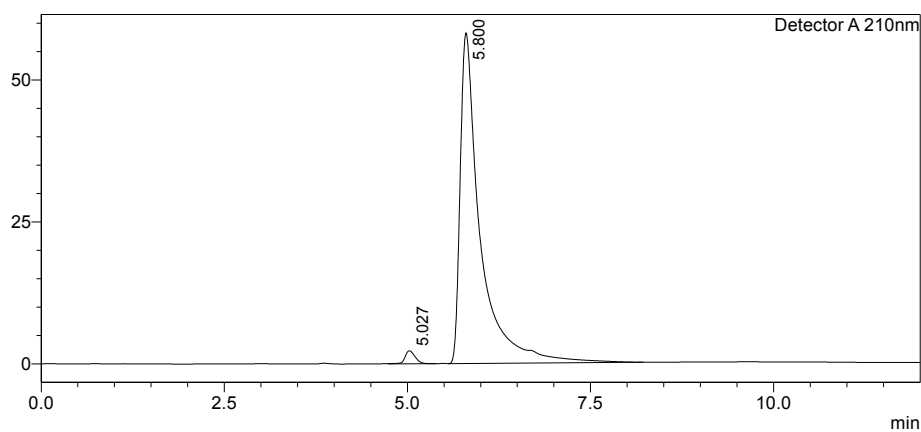
**ProPagen 14a**

Sample Name : NDFN-Me-001  
 Sample ID : NDFN-Me-001  
 Data Filename : XQ-NDFN-Me-002.lcd  
 Method Filename : method diamidate.lcm  
 Batch Filename : Batch02 diamidate 110219.lcb  
 Vial # : 1-1  
 Injection Volume : 5 uL  
 Date Acquired : 11/02/2019 14:48:12  
 Date Processed : 11/02/2019 15:00:14

Sample Type : Unknown  
 Acquired by : System Administrator  
 Processed by : System Administrator

## <Chromatogram>

mV



## <Peak Table>

Detector A 210nm				
Peak#	Ret. Time	Area	Height	Area%
1	5.027	22379	2294	1.976
2	5.800	1110111	58194	98.024
Total		1132490	60488	100.000

# Analysis Report

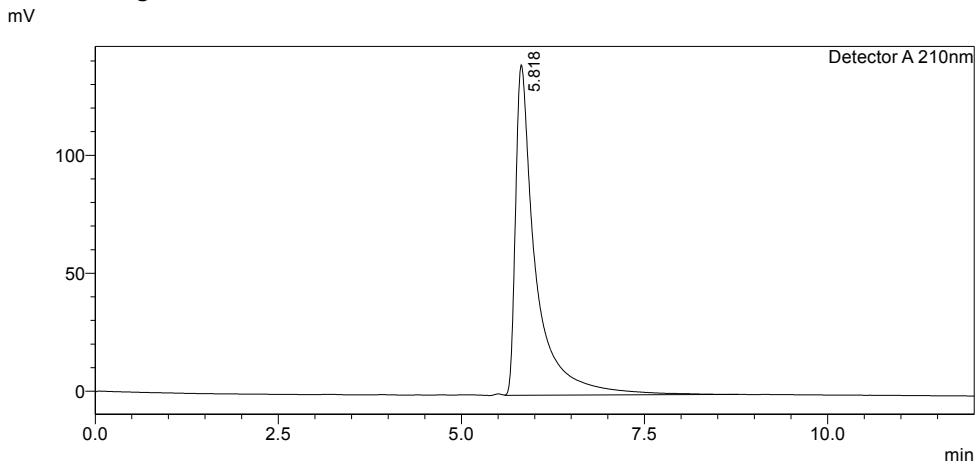
**<Sample Information>**

**ProPagen 14b**

Sample Name : NDFN-Iso001  
 Sample ID : Iso001  
 Data Filename : XQ-NDFN-ISO-01.lcd  
 Method Filename : method diamidate.lcm  
 Batch Filename : Batch01 diamidate 040219.lcb  
 Vial # : 1-2  
 Injection Volume : 10 uL  
 Date Acquired : 04/02/2019 14:37:18  
 Date Processed : 04/02/2019 14:49:19

Sample Type : Unknown  
 Acquired by : System Administrator  
 Processed by : System Administrator

**<Chromatogram>**



**<Peak Table>**

Detector A 210nm				
Peak#	Ret. Time	Area	Height	Area%
1	5.818	2693580	140021	100.000
Total		2693580	140021	100.000

# Analysis Report

## <Sample Information>

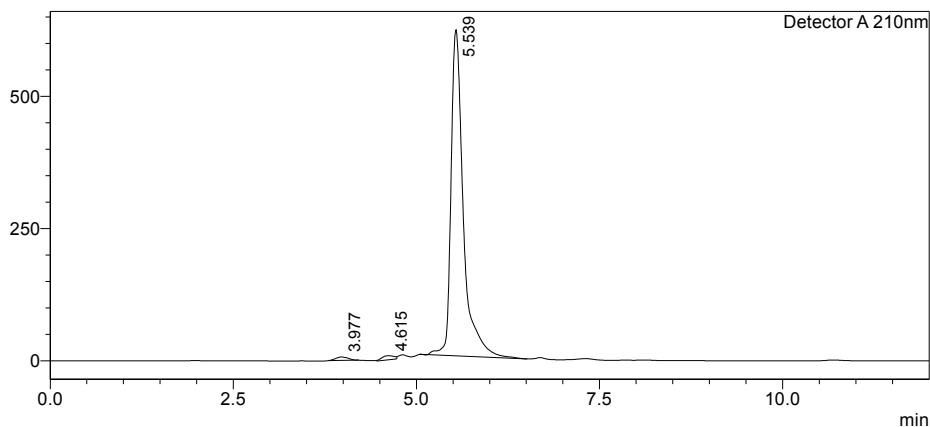
Sample Name : NDFN-tBu-001  
 Sample ID : NDFN-tBu-001  
 Data Filename : XQ-NDFN-tBu-003.lcd  
 Method Filename : method diamidate.lcm  
 Batch Filename : batch4 diamidate 110219.lcb  
 Vial # : 1-1  
 Injection Volume : 10 uL  
 Date Acquired : 11/02/2019 15:23:12  
 Date Processed : 11/02/2019 15:40:57

**ProPagen 14c**

Sample Type : Unknown  
 Acquired by : System Administrator  
 Processed by : System Administrator

## <Chromatogram>

mV



## <Peak Table>

Peak#	Ret. Time	Area	Height	Area%
1	3.977	76494	6656	1.035
2	4.615	92632	7718	1.253
3	5.539	7223345	616035	97.712
Total		7392471	630409	100.000

C:\LabSolutions\Data\YM\XQ\XQ-NDFN-tBu-003.lcd



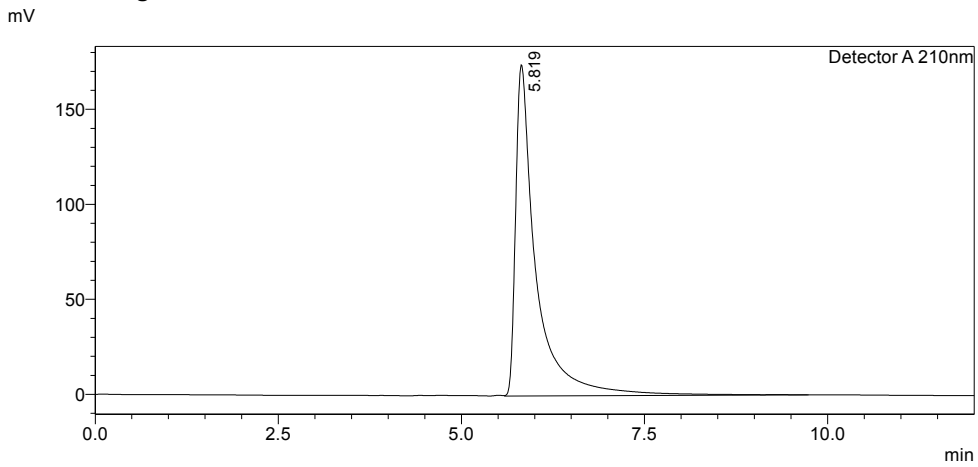
# Analysis Report

## <Sample Information>

**ProPagen 14d**

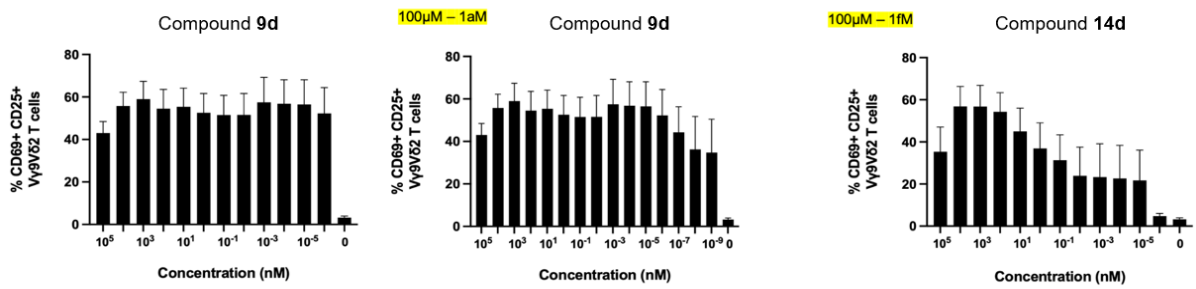
Sample Name : NDFN-Bn001  
Sample ID : Bn-01  
Data Filename : XQ-NDFN-Bn001.lcd  
Method Filename : method diamidate.lcm  
Batch Filename : Batch01 diamidate 050219.lcb  
Vial # : 1-3  
Injection Volume : 10 uL  
Date Acquired : 05/02/2019 12:50:17  
Date Processed : 05/02/2019 13:02:18  
Sample Type : Unknown  
Acquired by : System Administrator  
Processed by : System Administrator

## <Chromatogram>



## <Peak Table>

Detector A 210nm				
Peak#	Ret. Time	Area	Height	Area%
1	5.819	3388393	174211	100.000
Total		3388393	174211	100.000



**Figure S2.** Phosphonodiamidate ProPAgen 9d- and 14d-mediated activation of Vγ9/Vδ2 T cells after overnight incubation of PBMC with 9d at 1 attomolar (aM) to 100μM and 14d at 1 femtomolar (fM) to 100μM in order to determine the EC<sub>50</sub> values of these compounds. The level of activation is presented as % CD69+ CD25+ Vγ9/Vδ2 T cells. Data was analyzed with GraphPad Prism v9 software and is shown as mean ± SE (n=4).

**Figure S3. HPLC traces of ProPagens 9d and 14d following dissolution and storage.**

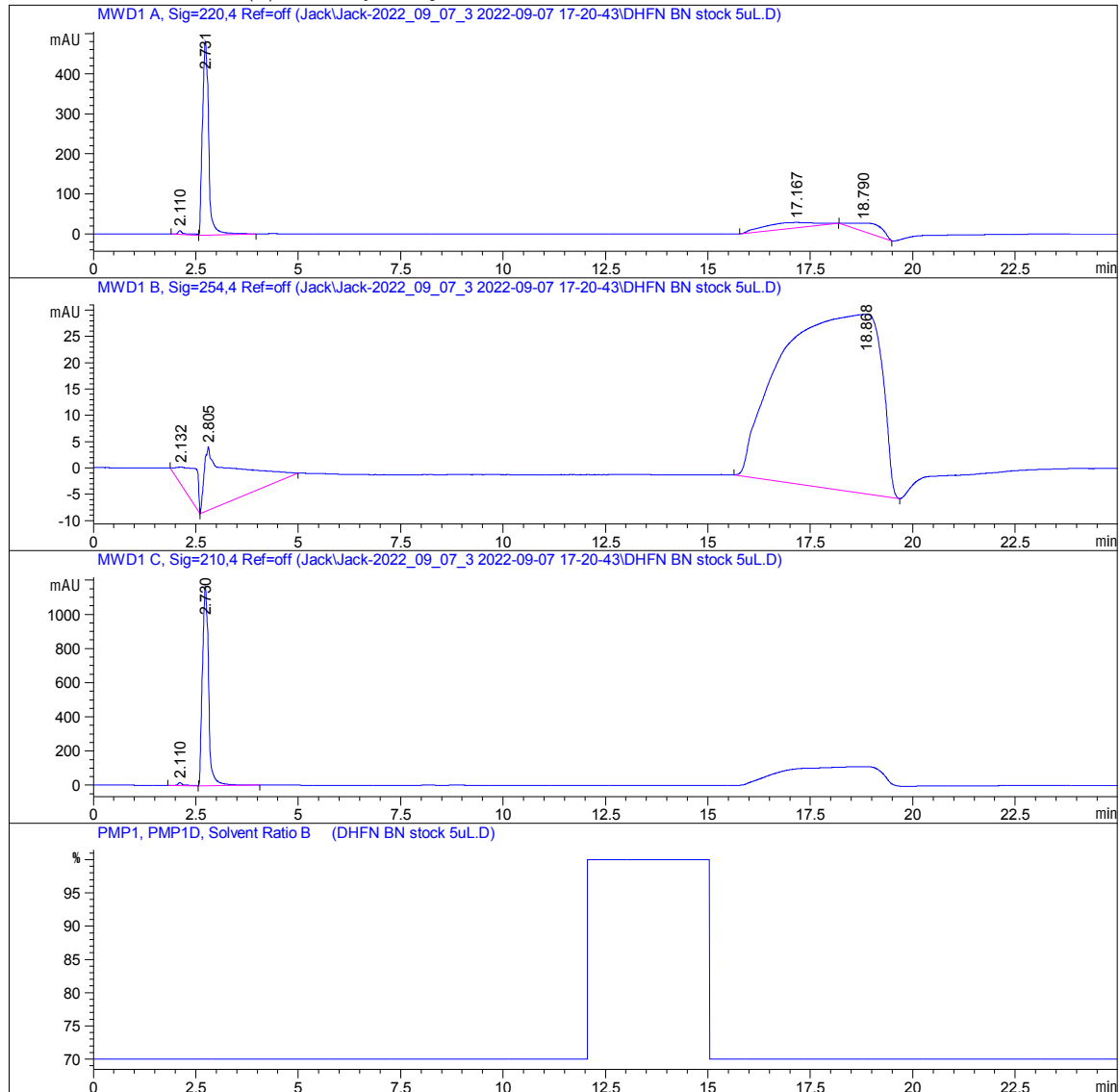
Data File C:\Chem32\3\Data\Jack\Jack-2022\_09\_07\_3 2022-09-07 17-20-43\DHFN BN stock 5uL.D  
 Sample Name: DHFN BN stock 5uL

```

=====
Acq. Operator   : SYSTEM                               Seq. Line :    4
Acq. Instrument : LC-FC                               Location  :    2
Injection Date  : 07-Sep-22 6:39:51 PM                Inj       :    1
                                                    Inj Volume: 15.000 µl
Different Inj Volume from Sample Entry! Actual Inj Volume : 5.000 µl
Method         : C:\Chem32\3\Data\Jack\Jack-2022_09_07_3 2022-09-07 17-20-43\7030 MeOHH2O
              : 12min.M (Sequence Method)
Last changed   : 05-Sep-22 10:33:20 AM by SYSTEM
Sample Info    : DHFN BN stock 5uL
  
```

**ProPagen 9d**

Additional Info : Peak(s) manually integrated



=====  
 Fraction Information  
 =====

=====  
No Fractions found.  
=====

=====  
Area Percent Report  
=====

**ProPagen 9d**

Sorted By : Signal  
Multiplier : 1.0000  
Dilution : 1.0000  
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=220,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.110	BB	0.1661	121.78243	9.72608	1.5731
2	2.731	BB	0.1415	5120.33057	483.71109	66.1404
3	17.167	BB	1.1526	1311.39526	13.34365	16.9396
4	18.790	BB	0.6911	1188.10461	20.29043	15.3470

Totals : 7741.61288 527.07125

Signal 2: MWD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.132	BB	0.6362	172.38980	3.27453	2.6891
2	2.805	BB	0.6326	642.50555	12.07816	10.0225
3	18.868	BB	1.9244	5595.75146	34.13763	87.2884

Totals : 6410.64682 49.49033

Signal 3: MWD1 C, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.110	BB	0.1220	154.47354	17.67632	1.2531
2	2.730	BB	0.1421	1.21732e4	1162.92981	98.7469

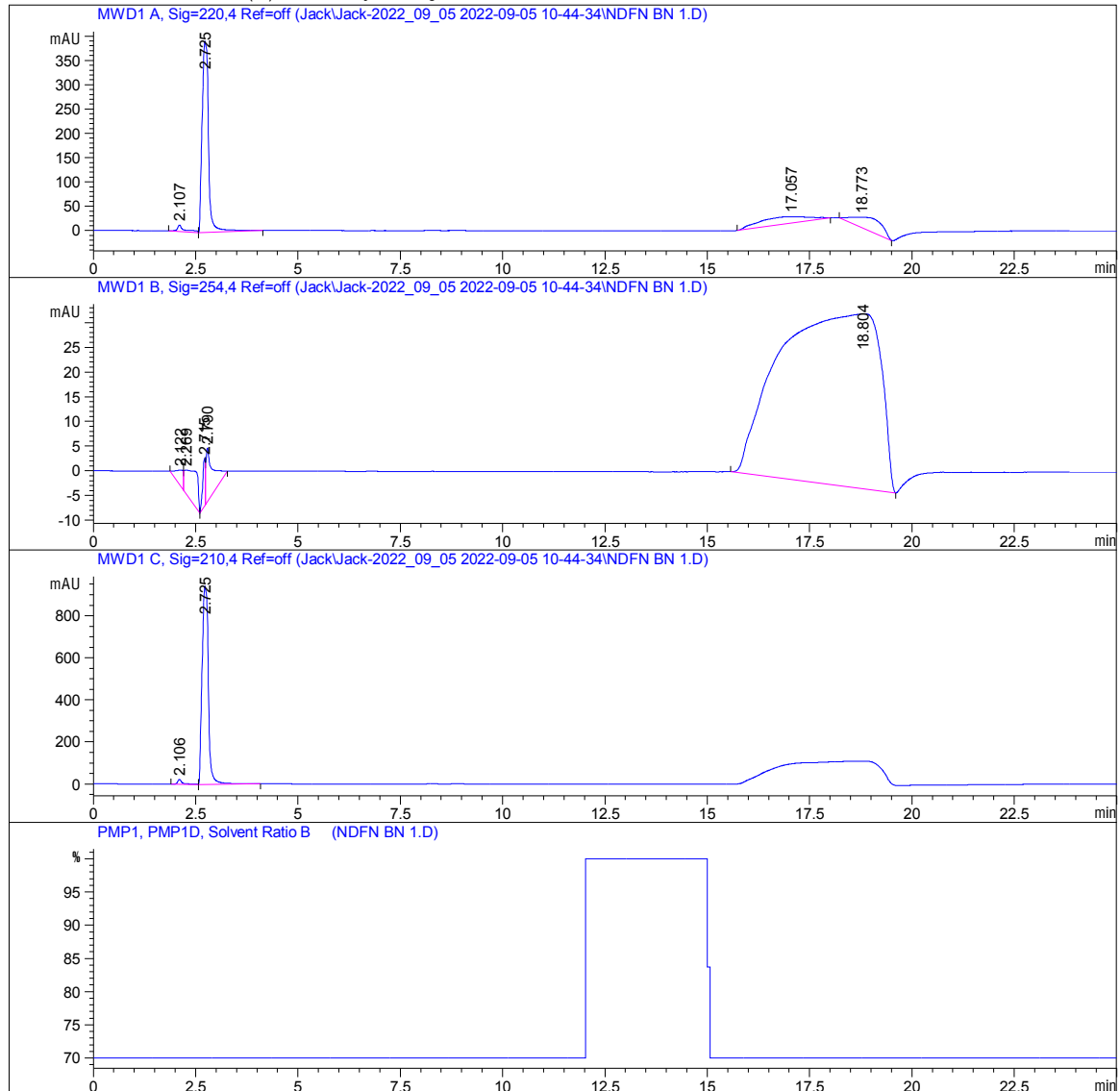
Totals : 1.23277e4 1180.60613

=====  
\*\*\* End of Report \*\*\*

```
=====
Acq. Operator   : SYSTEM                      Seq. Line :    8
Acq. Instrument : LC-FC                      Location  :   13
Injection Date  : 05-Sep-22 1:47:27 PM      Inj       :    1
                                           Inj Volume: 15.000 µl
Different Inj Volume from Sample Entry! Actual Inj Volume : 5.000 µl
Method         : C:\Chem32\3\Data\Jack\Jack-2022_09_05 2022-09-05 10-44-34\7030 Me0HH2O
                                           12min.M (Sequence Method)
Last changed   : 05-Sep-22 10:33:20 AM by SYSTEM
Sample Info    : NDFN BN 1
=====
```

**ProPagen 14d**

Additional Info : Peak(s) manually integrated



=====  
 Fraction Information

=====  
No Fractions found.  
=====

=====  
Area Percent Report  
=====

**ProPagen 14d**

Sorted By : Signal  
Multiplier : 1.0000  
Dilution : 1.0000  
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=220,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.107	BB	0.1696	167.34492	13.04740	2.4087
2	2.725	BV R	0.1439	4313.51758	393.42148	62.0863
3	17.057	BV R	1.1181	1212.06775	12.80741	17.4458
4	18.773	BB	0.7254	1254.68115	20.51801	18.0592

Totals : 6947.61140 439.79429

Signal 2: MWD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.122	BV	0.1744	40.23585	3.08034	0.6442
2	2.269	VB	0.3392	130.60985	4.77356	2.0911
3	2.715	BV	0.0656	42.26519	9.54859	0.6767
4	2.790	VB	0.1572	132.64157	10.80398	2.1236
5	18.804	BB	1.9574	5900.34521	35.38522	94.4645

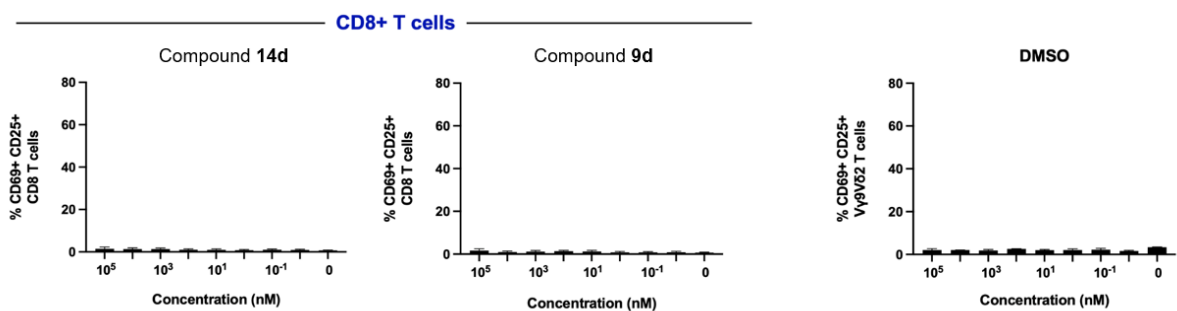
Totals : 6246.09768 63.59169

Signal 3: MWD1 C, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.106	BB	0.1224	203.74788	23.20883	1.9814
2	2.725	BB	0.1406	1.00791e4	943.64679	98.0186

Totals : 1.02828e4 966.85562

=====  
\*\*\* End of Report \*\*\*



**Figure S4.** Phosphonodiamidate ProPAgen 9d- and 14d-mediated activation of CD8+ T cells (left) in the same overnight cultures as Vγ9/Vδ2 T cells (mean ± SE; n=4). Level of Vγ9/Vδ2 T cell activation with DMSO (right) diluted in the same way as the ProPAgens (mean ± SE; n=4). Data was analyzed with GraphPad Prism v9 software.