Electronic Supplementary Material (ESI) for Analyst.

Competitive ELISA for N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) determination in human plasma

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Figure S1. Effect of the Tween effect (Top-left), ionic strength (Top-right), pH (Bottom-left) and the preincubation time (Bottom-right) of the buffered media on the As251/NB1-SIA-BSA immunoassay features.. The IC50 is expressed in nM. (●, IC_{50}), (■, A_{max}), (①, A_{max}/IC_{50}). Left axis, IC50, Right axis Amax, Amax/IC50

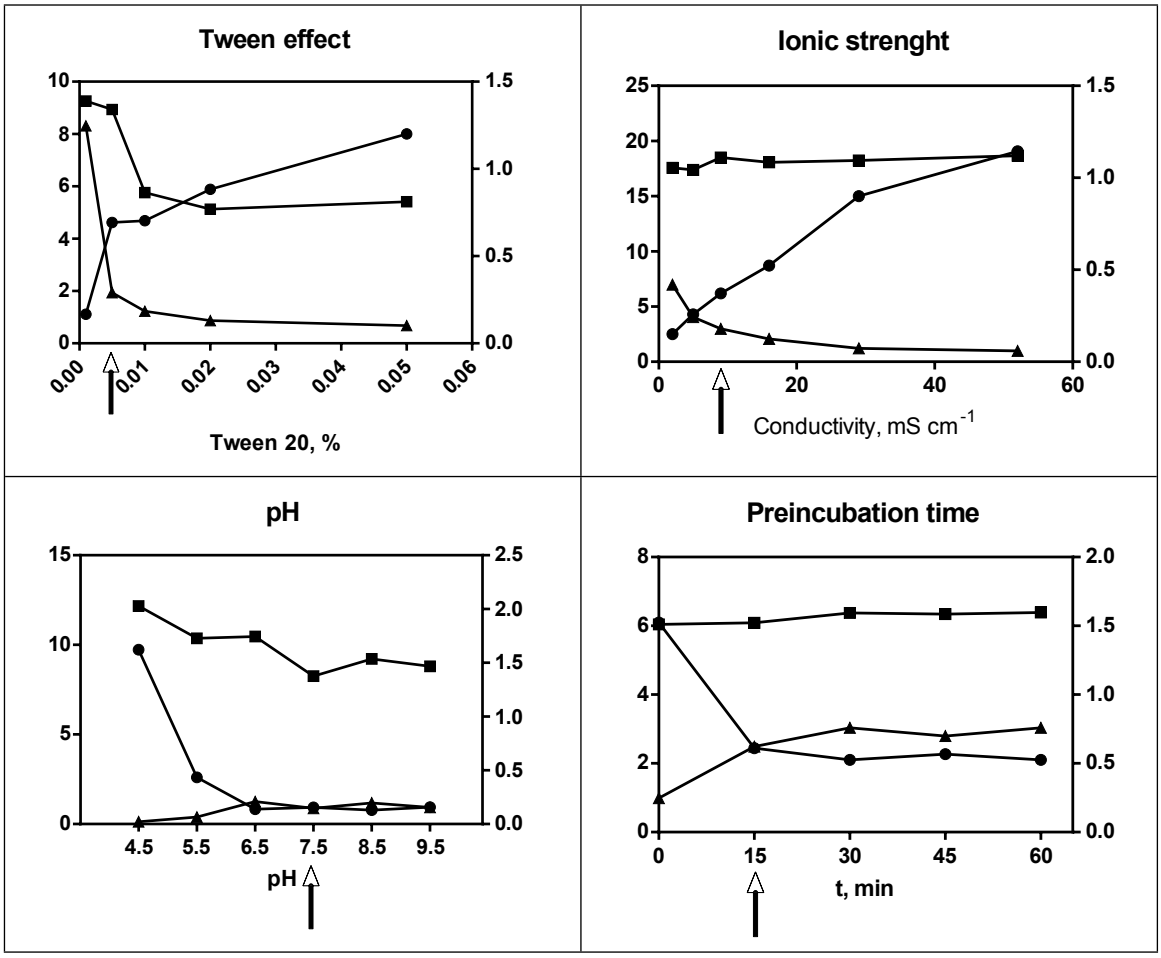
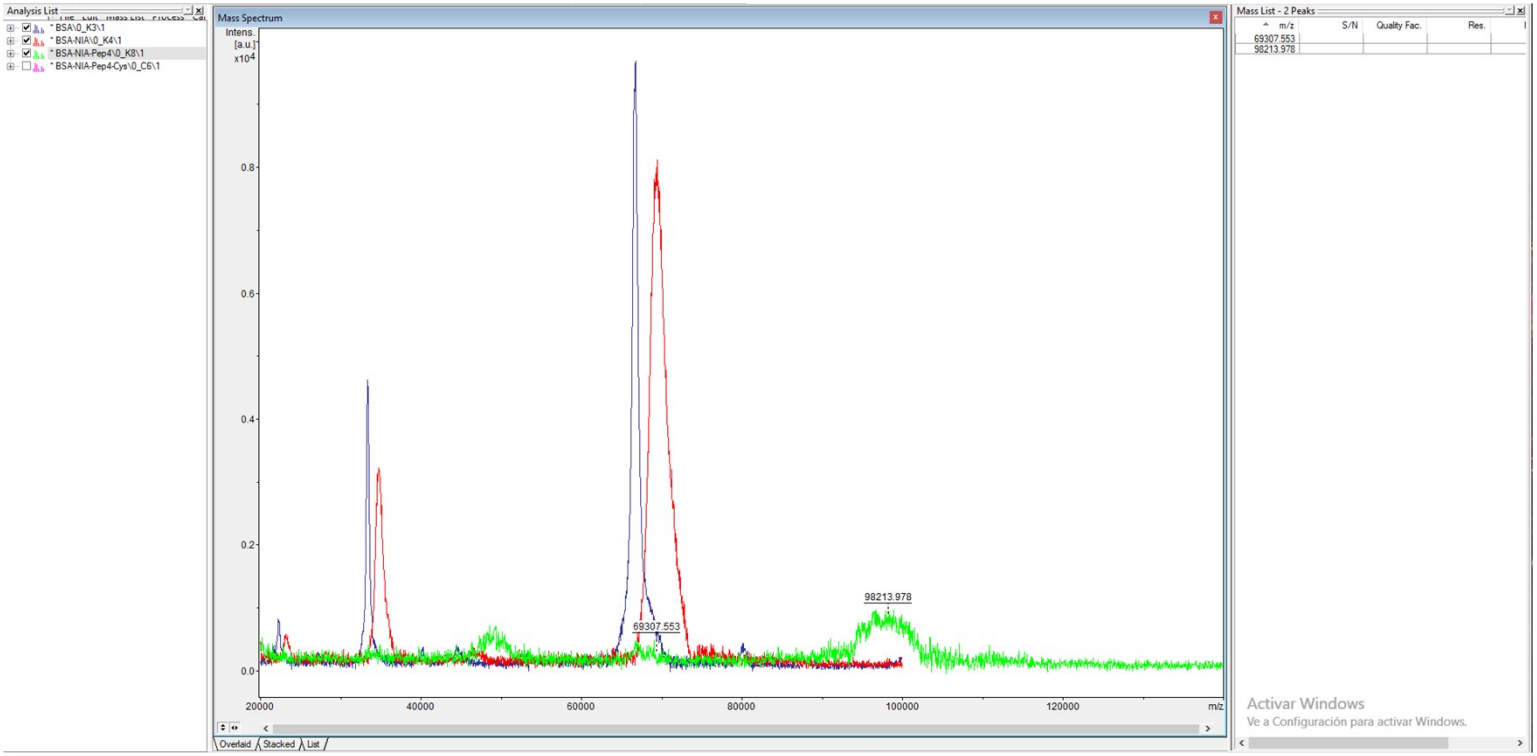
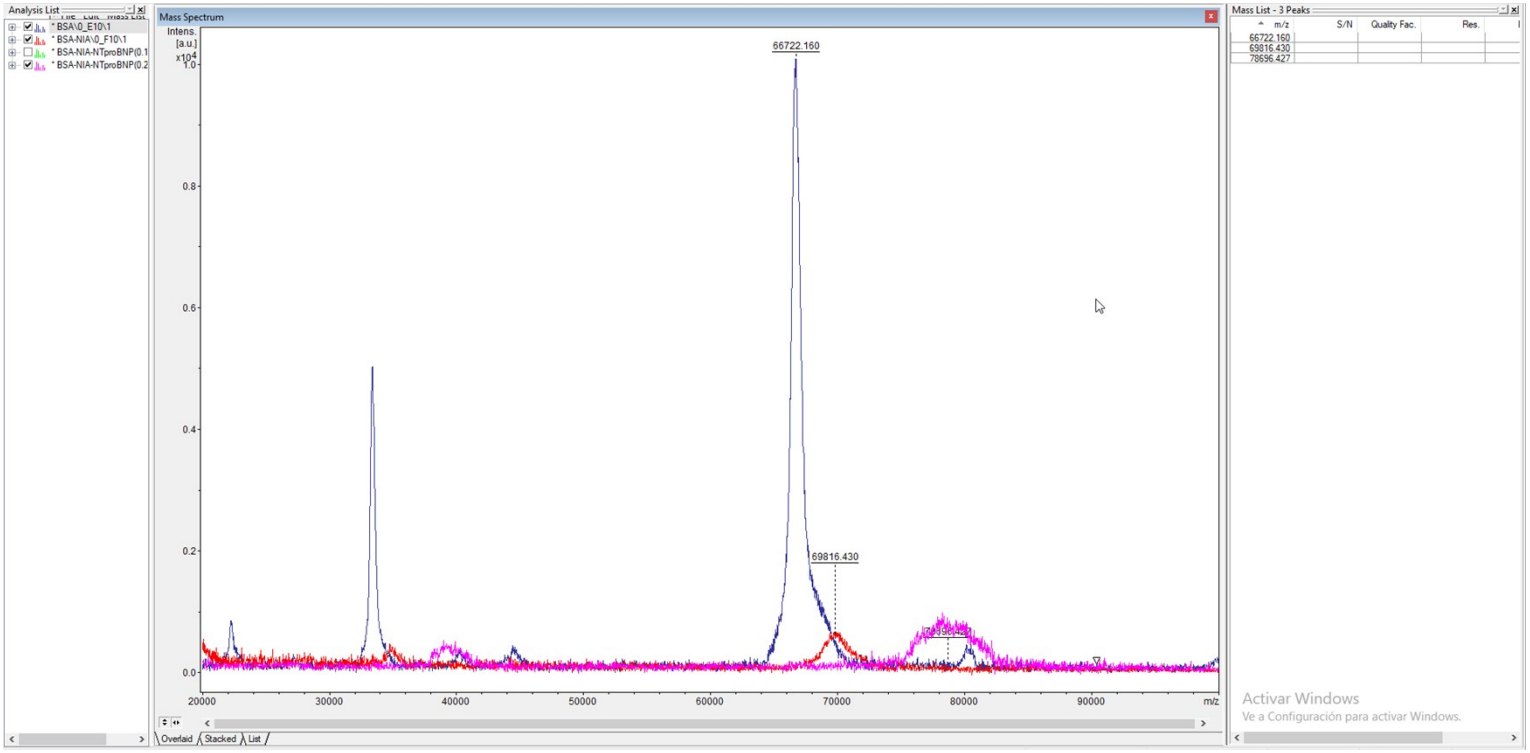


Figure S2. MALDI-TOF MS spectra from the BSA conjugates prepared

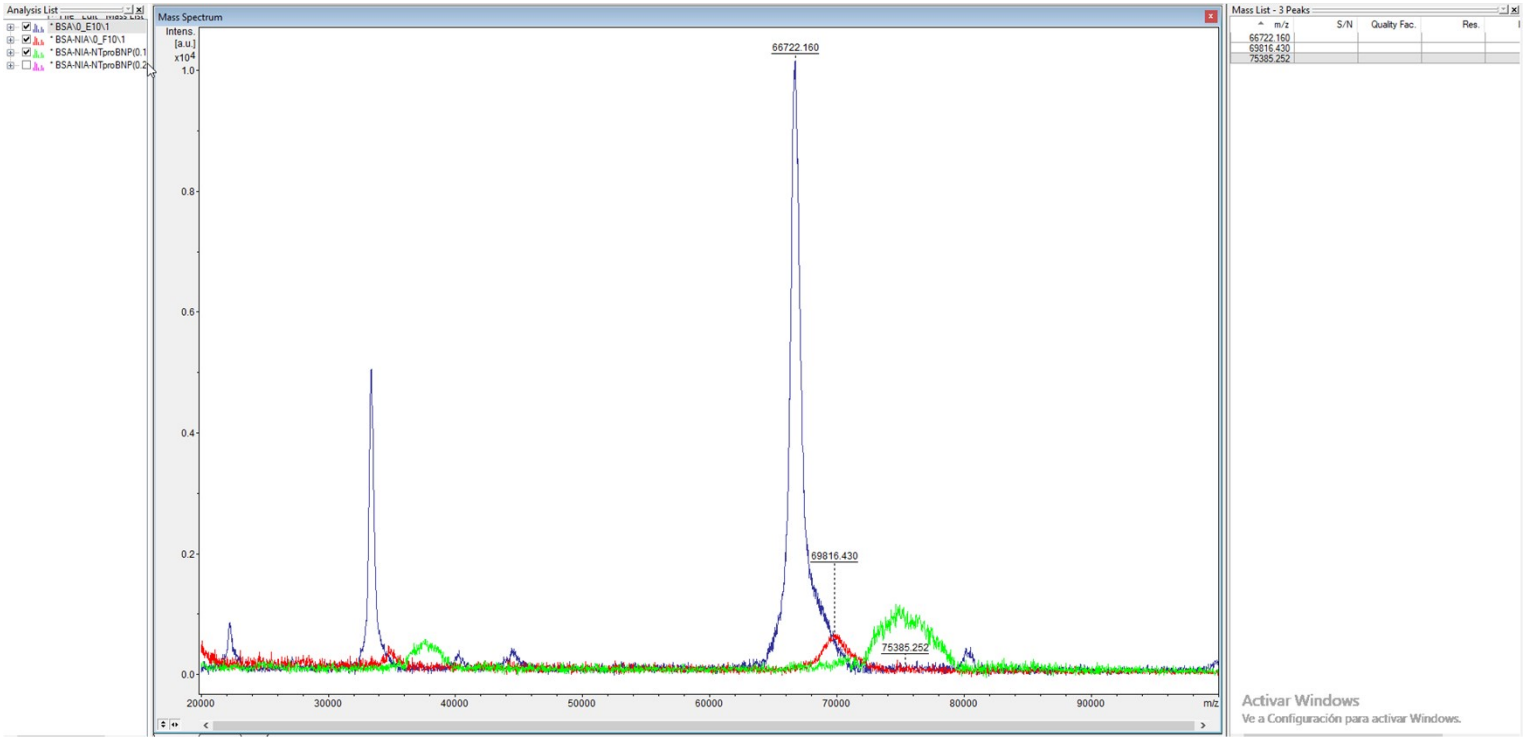
NB1-SIA-BSA (1:2:2)



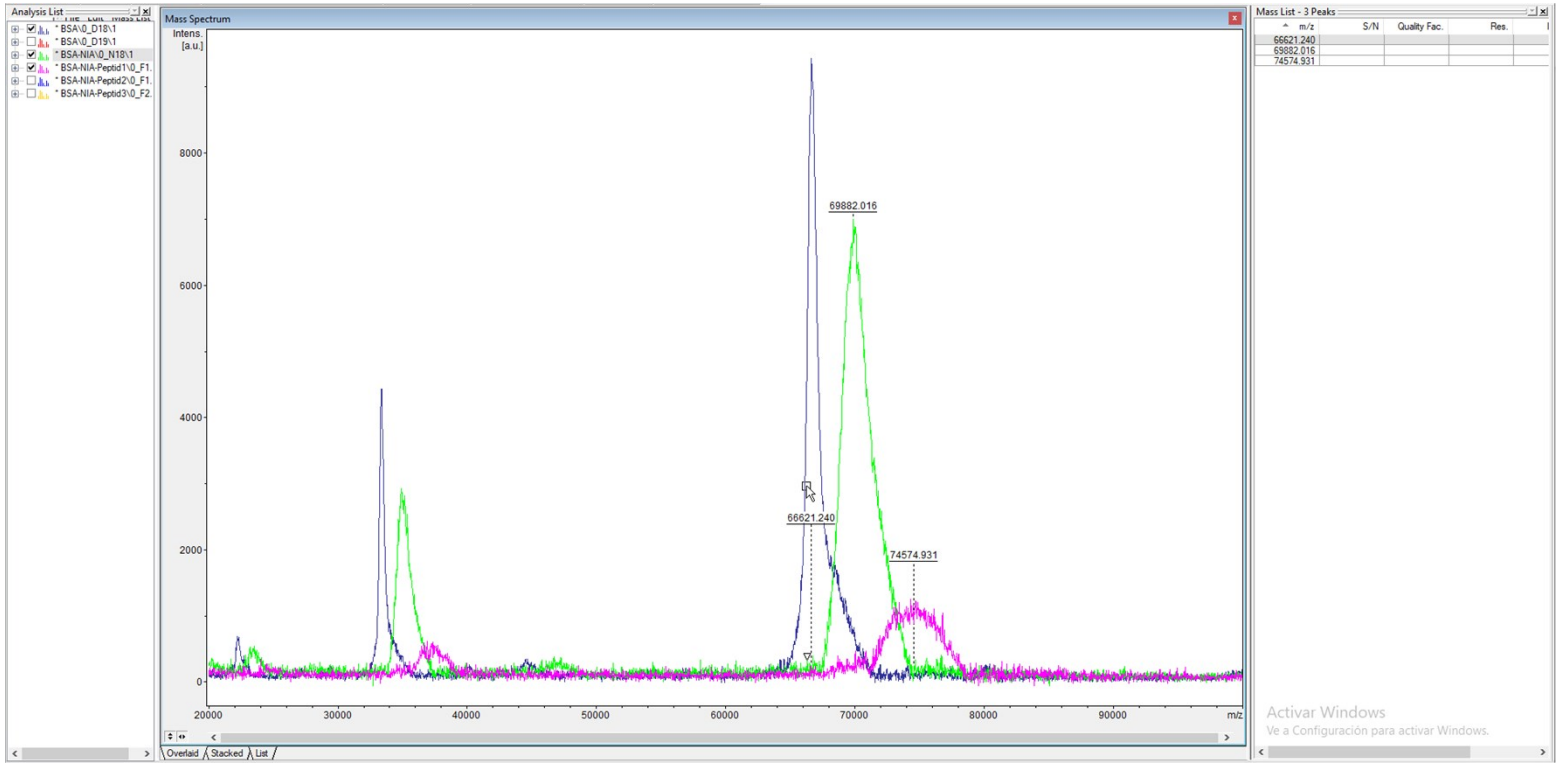
NB1-SIA-BSA (1:2:0.2)



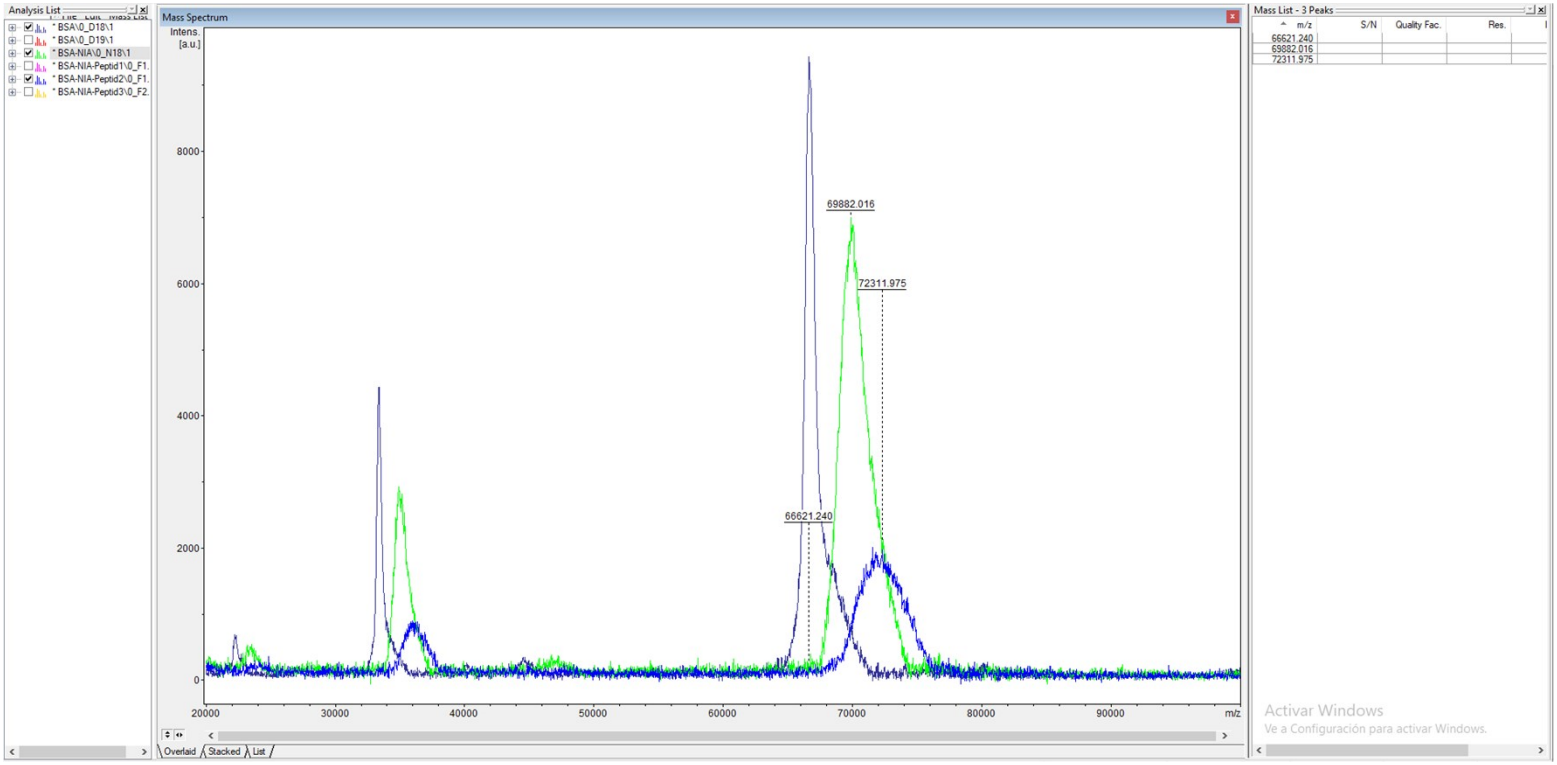
NB1-SIA-BSA (1:2:0.1)



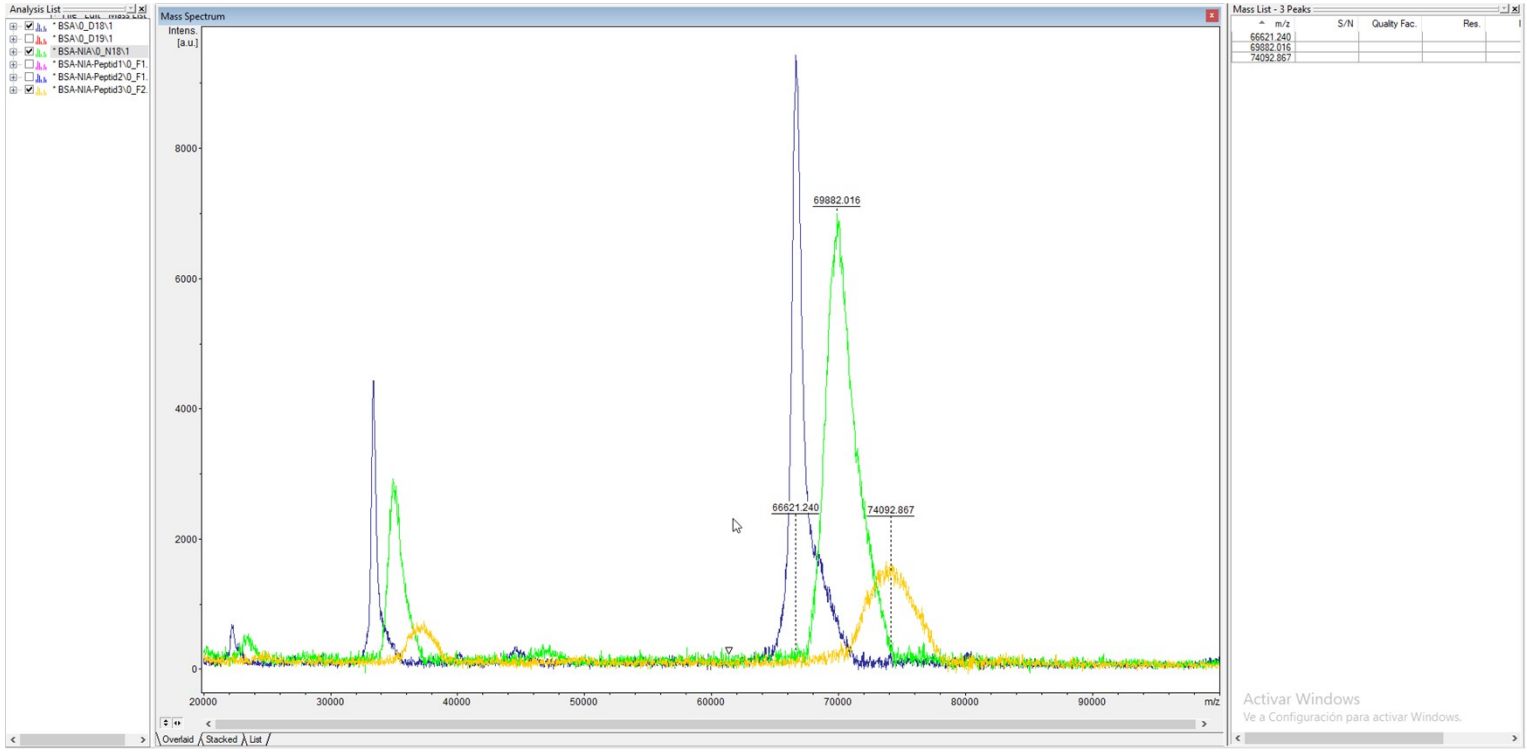
NB2-SIA-BSA (1:2:0.1)



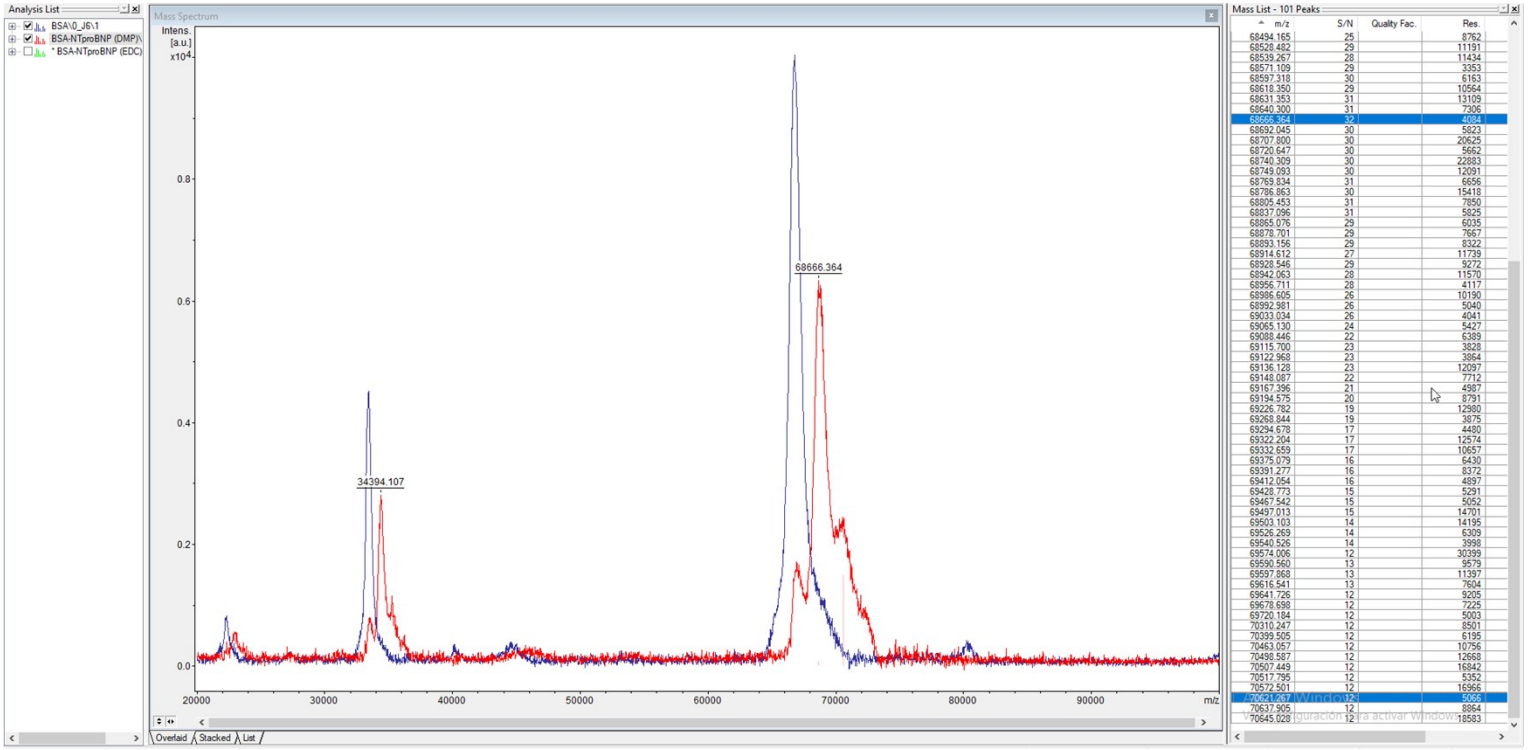
NB3-SIA-BSA (1:2:0.1)



NB4-SIA-BSA (1:2:0.1)



NB1-DMP-BSA


Analysis List
BSA\0_J6\1
BSA-NTproBNP (DMP)\
* BSA-NTproBNP (EDC)
Mass Spectrum
Intens.
[a.u.]
x10^4
0.8
0.6
0.4
0.2
0.0
34394.107
68666.364
20000
30000
40000
50000
60000
70000
80000
90000
m/z
Overlaid
Stacked
List
Mass List - 101 Peaks
m/z
S/N
Quality Fac.
Res.
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68528.482 29 11191
68539.267 28 11434
68571.109 29 3353
68597.318 30 6163
68618.350 29 10564
68631.353 31 13109
68640.300 31 7306
68668.364 32 4004
68692.045 30 5823
68707.800 30 20625
68720.647 30 5662
68740.309 30 22883
68749.093 30 12091
68769.834 31 6656
68786.863 30 15418
68805.453 31 7850
68837.096 31 5825
68865.076 29 6035
68878.701 29 7667
68893.156 29 8322
68914.612 27 11739
68928.546 29 9272
68942.063 28 11570
68956.711 28 4117
68986.605 26 10190
68992.981 26 5040
69033.034 26 4041
69065.130 24 5427
69088.446 22 6389
69115.700 23 3828
69122.968 23 3864
69136.128 23 12097
69148.087 22 7712
69167.396 21 4987
69194.575 20 8791
69226.782 19 12980
69268.844 19 3875
69294.678 17 4480
69322.204 17 12574
69332.659 17 10657
69375.079 16 6430
69391.277 16 8372
69412.054 16 4897
69428.773 15 5291
69467.542 15 5052
69497.013 15 14701
69503.103 14 14195
69526.269 14 6309
69540.526 14 3998
69574.006 12 30399
69590.560 13 9579
69597.868 13 11397
69616.541 13 7604
69641.726 12 9205
69678.898 12 7225
69720.184 12 5003
70310.247 12 8501
70399.505 12 6195
70463.057 12 10756
70498.587 12 12668
70507.449 12 16842
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70572.501 12 16966
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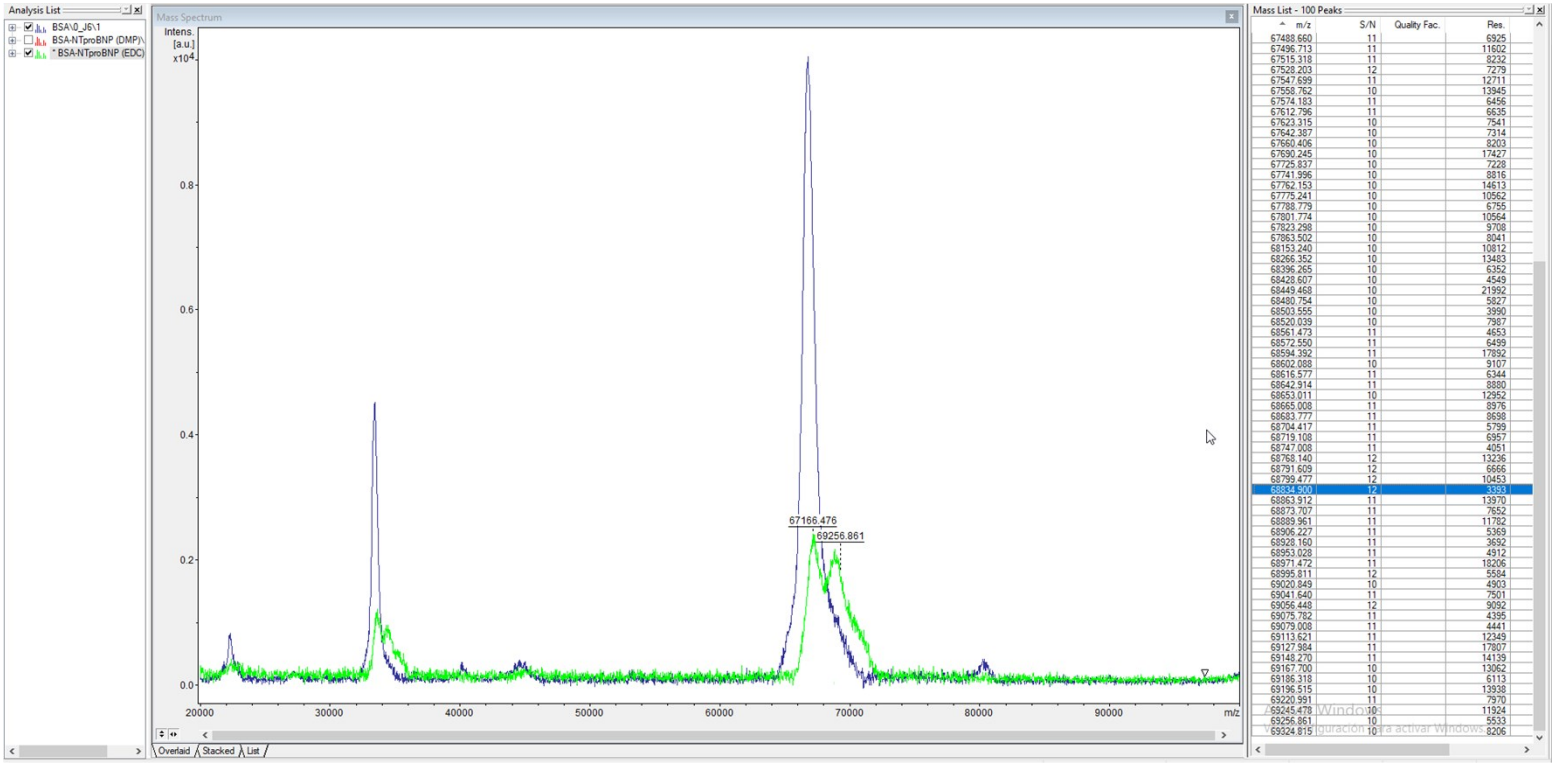
70645.028 12 18583

NB1-EDC-BSA


Analysis List
BSA\0_J6\1
BSA-NTproBNP (DMP)\
* BSA-NTproBNP (EDC)
Mass Spectrum
Intens.
[a.u.]
x10^4
0.8
0.6
0.4
0.2
0.0
67166.476
69256.861
20000
30000
40000
50000
60000
70000
80000
90000
m/z
Overlaid
Stacked
List
Mass List - 100 Peaks
m/z
S/N
Quality Fac.
Res.
67488.660 11 6925
67496.713 11 11602
67515.318 11 8232
67528.203 12 7279
67547.699 11 12711
67558.762 10 13945
67574.183 11 6456
67612.796 11 6635
67623.315 10 7541
67642.367 10 7314
67660.406 10 8203
67690.245 10 17427
67725.837 10 7228
67741.996 10 8816
67762.153 10 14613
67775.241 10 10562
67788.779 10 6755
67801.774 10 10564
67823.298 10 9708
67863.502 10 8041
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68266.352 10 13483
68396.265 10 6352
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68449.468 10 21992
68480.754 10 5827
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68791.609 12 6666
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68863.912 11 13970
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68906.227 11 5369
68928.160 11 3692
68953.028 11 4912
68971.472 11 18206
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69075.782 11 4395
69079.008 11 4441
69113.621 11 12349
69127.984 11 17807
69148.270 11 14139
69167.700 10 13062
69186.318 10 6113
69196.515 10 13938
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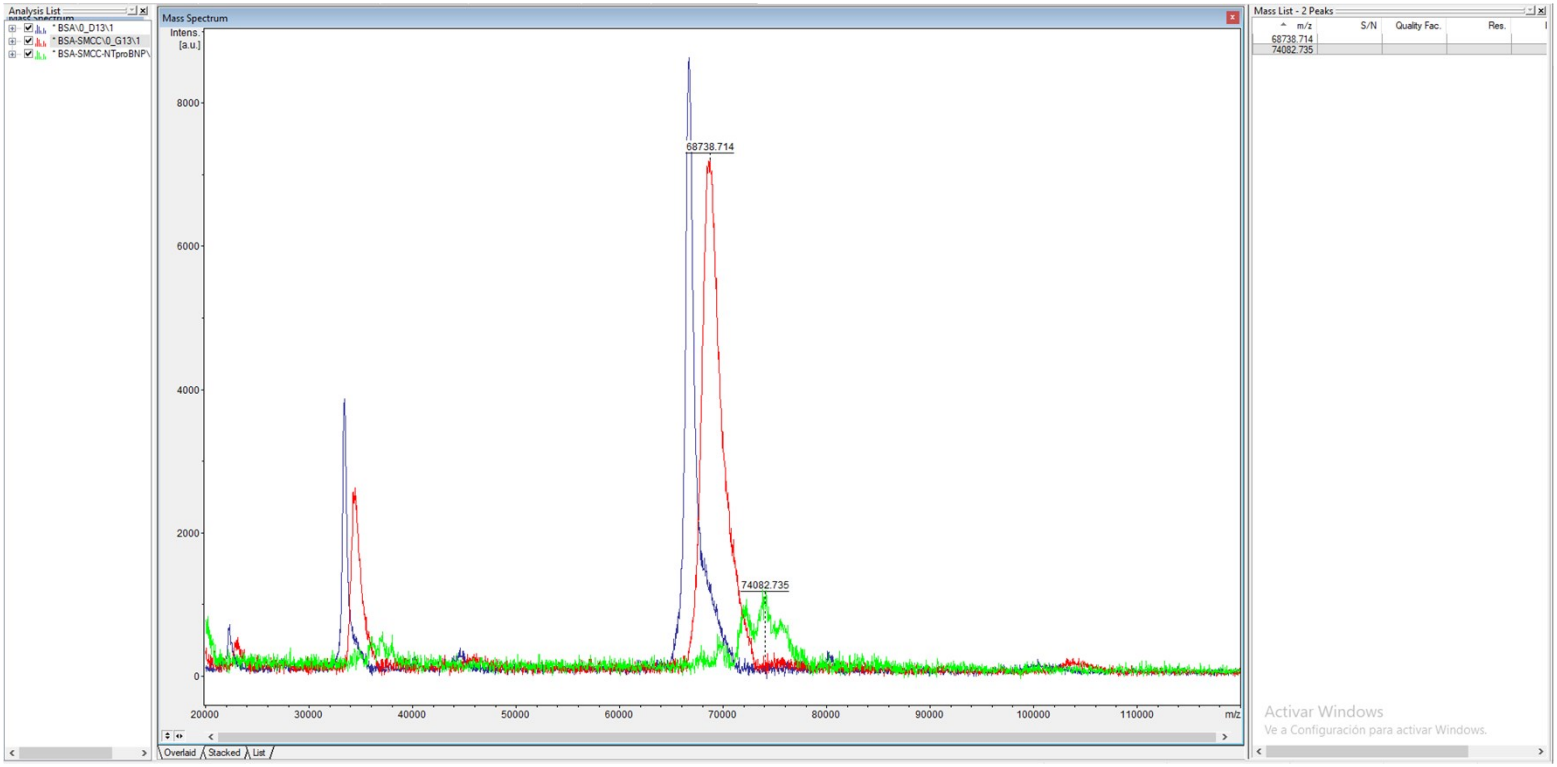
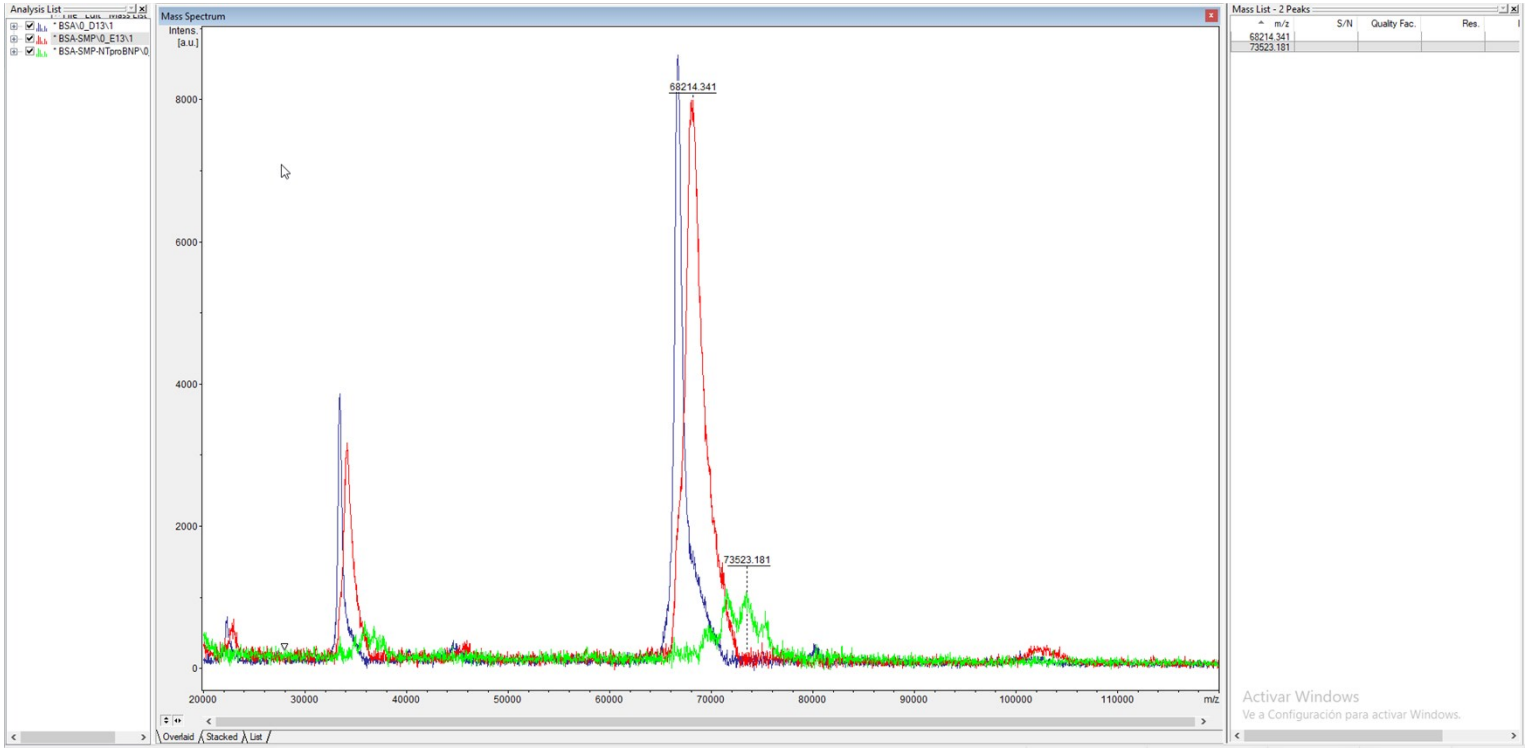
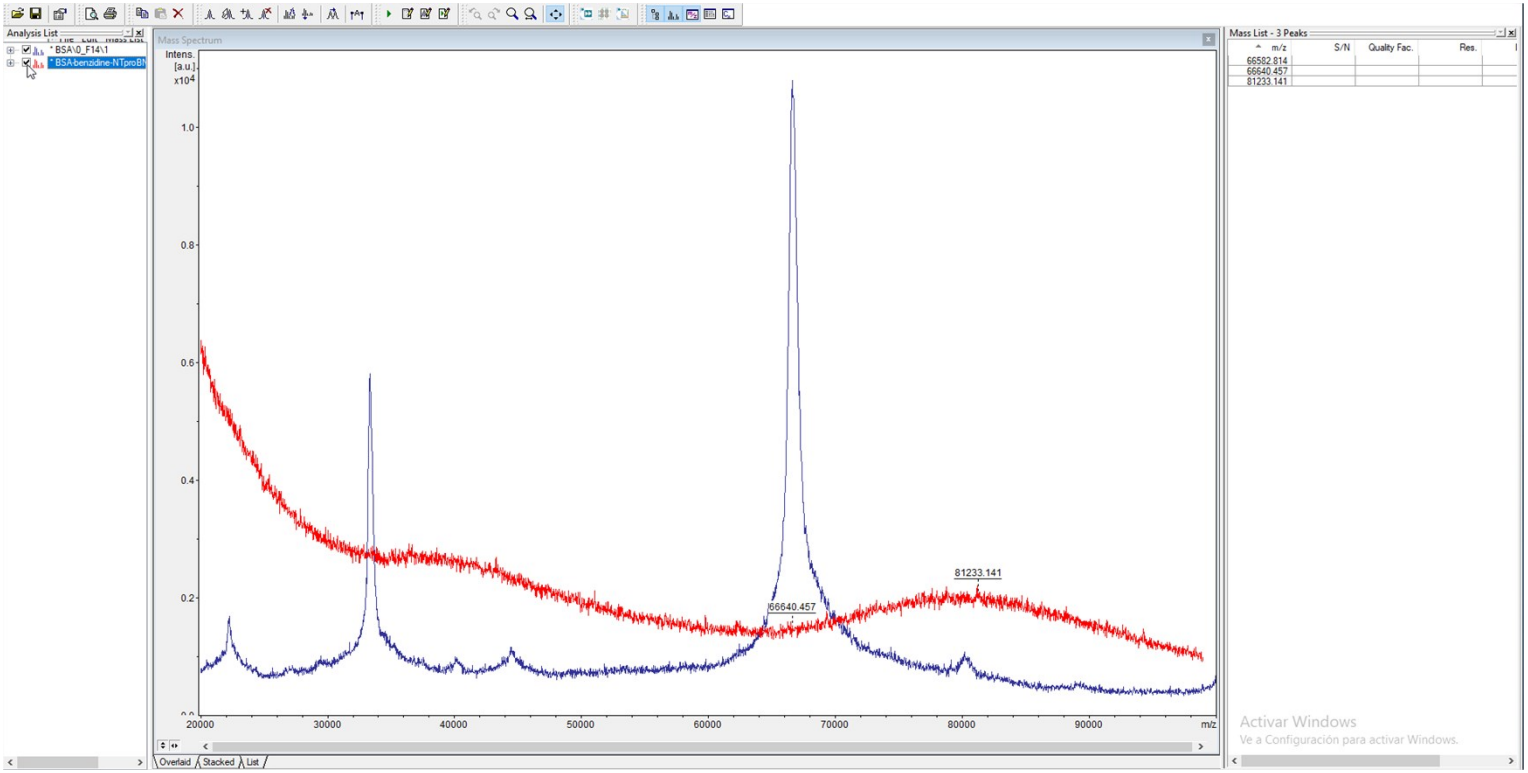
NB1-SMCC-BSA (1:0.32:0.32)



NB1-SMP-BSA (1:0.32:0.32)



NB1-BDB-BSA (1:5:1.44)



NB1-SMCC-HRP (1:4:4)

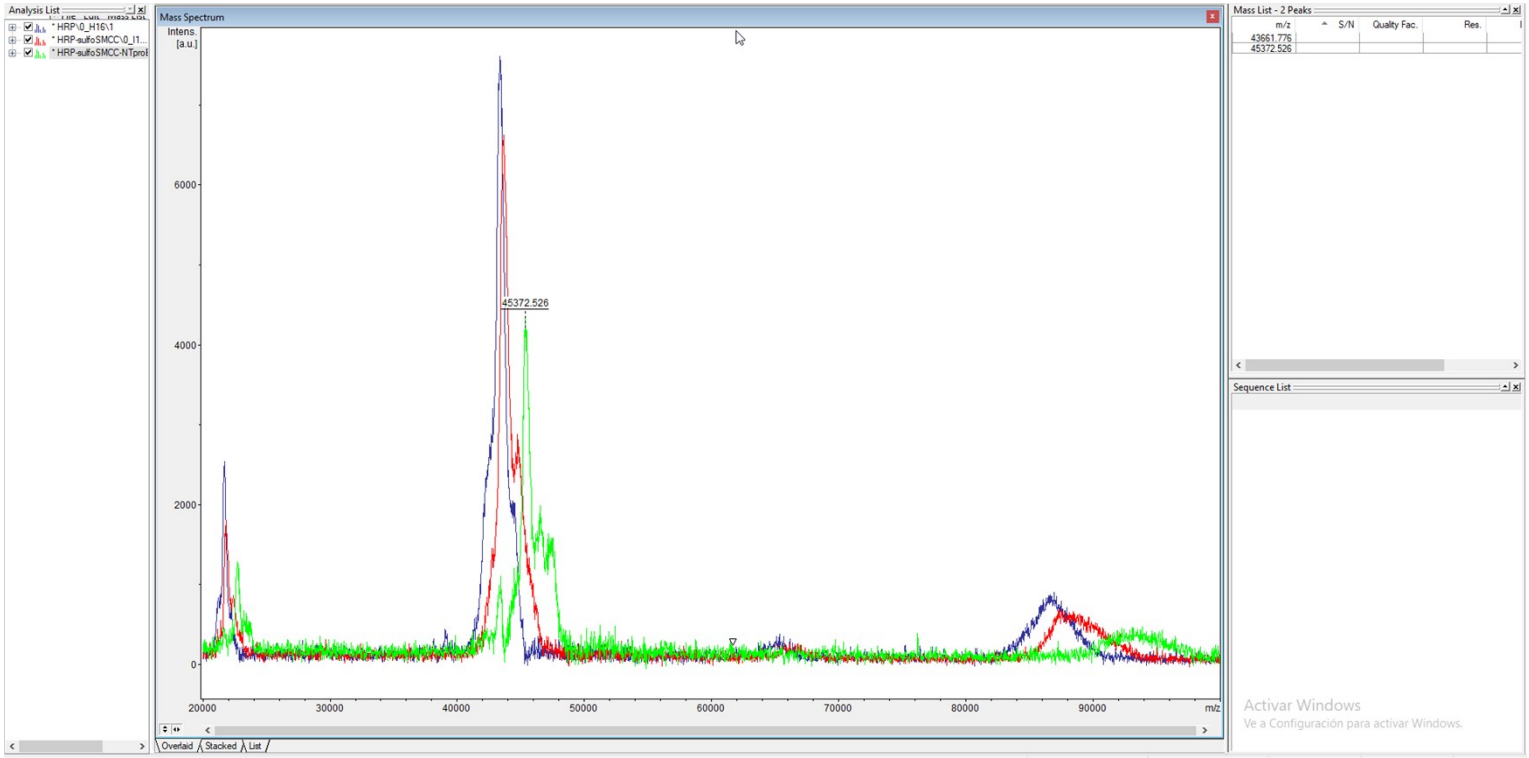


Table S1. Linker and peptide hapten densities(δ) for BSA or HRP conjugates. Peptide conjugation is calculated assuming that BSA has 30 accessible lysines residues whereas HRP has 2 accessible lysines

Immunoreagent	**δ linker**	**δ peptide**	**%conjugation**
NB1-SIA-BSA (1:2:2)	17	17	57
NB1-SIA-BSA (1:2:0.2)	19	5	17
NB1-SIA-BSA (1:2:0.1)	19	3	10
NB2-SIA-BSA (1:2:0.1)	19	3	10
NB3-SIA-BSA (1:2:0.1)	19	3	10
NB4-SIA-BSA (1:2:0.1)	19	4	13
NB1-EDC-BSA	11	1	3
NB1-DMP-BSA	8	1	3
NB1-SMP-BSA (1:0.32:0.32)	10	3	10
NB1-SMCC-BSA (1:0.32:0.32)	9	3	10
NB1-BDB-BSA (1:5:1.44)	-	8	27
NB1-SMCC-HRP (1:4:4)	1	1	50

Table S2. Analytical parameters of the calibration curve from NB1-SIA-BSA and NB1-sSMCC-HRP competition antigens with raised antibodies against NT-proBNP (As251-253). Each calibration curve was performed with two replicates

	A) NB1-SIA-BSA			**B) NB1-sSMCC-HRP**		
	As 251	**As 252**	**As 253**	**As 251**	**As 252**	**As 253**
Abs_{max}	0.85	1.08	0.79	-	0.55	0.82
Abs_{min}	0.11	0.66	0.37	-	0.30	0.24
IC50, ng mL^{-1}	34.03	270.40	837.70	-	79.93	202.50
Slope	-0.92	-2.34	-0.96	-	-1.04	-1.02
R^2	0.98	0.85	0.72	-	0.91	0.98

Table S3. Analytical parameters of the calibration curve from NB1-SIA-BSA/As251-253 depending on the hapten density (δ) of NB1-SIA-BSA conjugate. Each calibration curve was performed with two replicates

	As251/NB1-SIA-BSA		
	δ=17	**δ=5**	**δ=3**
Abs_{max}	0.83	1.23	0.92
Abs_{min}	0.13	0.13	0.10
Slope	-1.02	-0.81	-0.96
R2	0.99	0.99	0.99
IC50, ng mL-1	35.48	11.64	7.77
Working range, ng mL-1	6.67-119.54	2.04-66.37	1.83-33.89
LOD, ng mL-1	1.97	0.72	0.77

Table S4. Immunoassay features for the different competitors, depending on different crosslinkers nature and different location with As 251. Each calibration curve was performed with two replicates

	. **Cross-linker heterology**			**Location heterology**		
	SIA (δ=3)	**SMP** (δ=3)	**SMCC** (δ=3)	**EDC** (δ=1)	**DMP** (δ=1)	**BDB** (δ=8)
Abs_{max}	0.85	1.22	1.19	1.00	0.94	0.69
Abs_{min}	0.08	0.07	0.10	0.06	0.07	0.18
Slope	-1.07	-0.81	-0.94	-0.81	-0.87	-1.55
R2	0.99	0.99	1.00	0.99	0.99	0.98
IC50, ng mL-1	3.94	4.92	9.64	5.39	7.19	8.22
Working range, ng mL^{-1}	1.16–15.65	1.00–28.69	2.26–44.86	0.94–28.59	1.38–36.28	3.15–23.88
LOD, ng mL-1	0.57	0.41	0.96	0.33	0.51	1.64

Table S5. . Immunoassay features for the different competitors, depending on different composition heterology with As 251. Each calibration curve was performed with two replicates

	As251 /NB1-SIA-BSA	**As251 /NB2-SIA-BSA**	**As252 /NB2-SIA-BSA**	**As252 /NB4-SIA-BSA**	**As253 /NB2-SIA-BSA**	**As253 /NB4-SIA-BSA**
Abs_{max}	0.97	1.00	0.94	0.95	0.97	0.97
Abs_{min}	0.12	0.08	0.83	0.23	0.27	0.28
Slope	-1.07	-1.17	-1.35	-1.14	-0.94	-1.15
R2	0.98	0.994	0.60	0.97	0.98	0.97
IC50, ng mL-1	5.69	20.92	122.8	372.9	312.2	289.9