

Detecting M-Protein via Mass Spectrometry and Affinity Beads: Enrichment With Mixed Kappa-Lambda Beads Enables Prompt Application in Clinical Laboratories

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Supplemental Data Table S1. Molecular weight differences observed via high-resolution MS using daratumumab and cetuximab, treated with or without IAA

Sample	Average MW	Mass observed with high-resolution MS (DTT)	Difference in the average MW	Mass observed with high-resolution MS (DTT-IAA)	Difference in the average MW
1+ LC of daratumumab	23,384.0	23,384.4	0.4	23,441.8	57.8
1+ LC of cetuximab	23,427.0	23,427.4	0.4	23,483.4	56.4

Abbreviations: DTT, dithiothreitol; DTT-IAA, dithiothreitol with iodoacetamide treatment; IAA, iodoacetamide; 1+ LC, single-charged light chain; Melon-MALDI-TOF, Melon Kit with C4 ZipTip combined with matrix-assisted laser desorption/ionization time-of-flight; MS: mass spectrometry; MW: molecular weight.

Supplemental Data Table S2. Range and differences in the m/z ratios found during precision testing

Preparation method	Sample	LC charge	m/z range	
Melon Kit+C4 ZipTip	Normal	1+	23,271.8	23,356.1
	Abnormal		23,052.6	23,098.7
	Normal	2+	11,495.5	11,699.6
	Abnormal		11,390.4	11,596.6
MB	Normal	1+	23,305.3	23,387.9
	Abnormal		23,282.7	23,394.7
	Normal	2+	11,415.9	11,749.2
	Abnormal		11,412.2	11,791.2
NB	Normal	1+	23,277.3	23,444.6
	Abnormal		23,052.6	23,171.9
	Normal	2+	11,480.1	11,728.4
	Abnormal		11,389.1	11,610.8

Abbreviations: LC, light chain; MB, magnetic beads; NB, nanobody affinity beads.

Supplemental Data Table S3. CVs observed with five replicates tested in 1 day

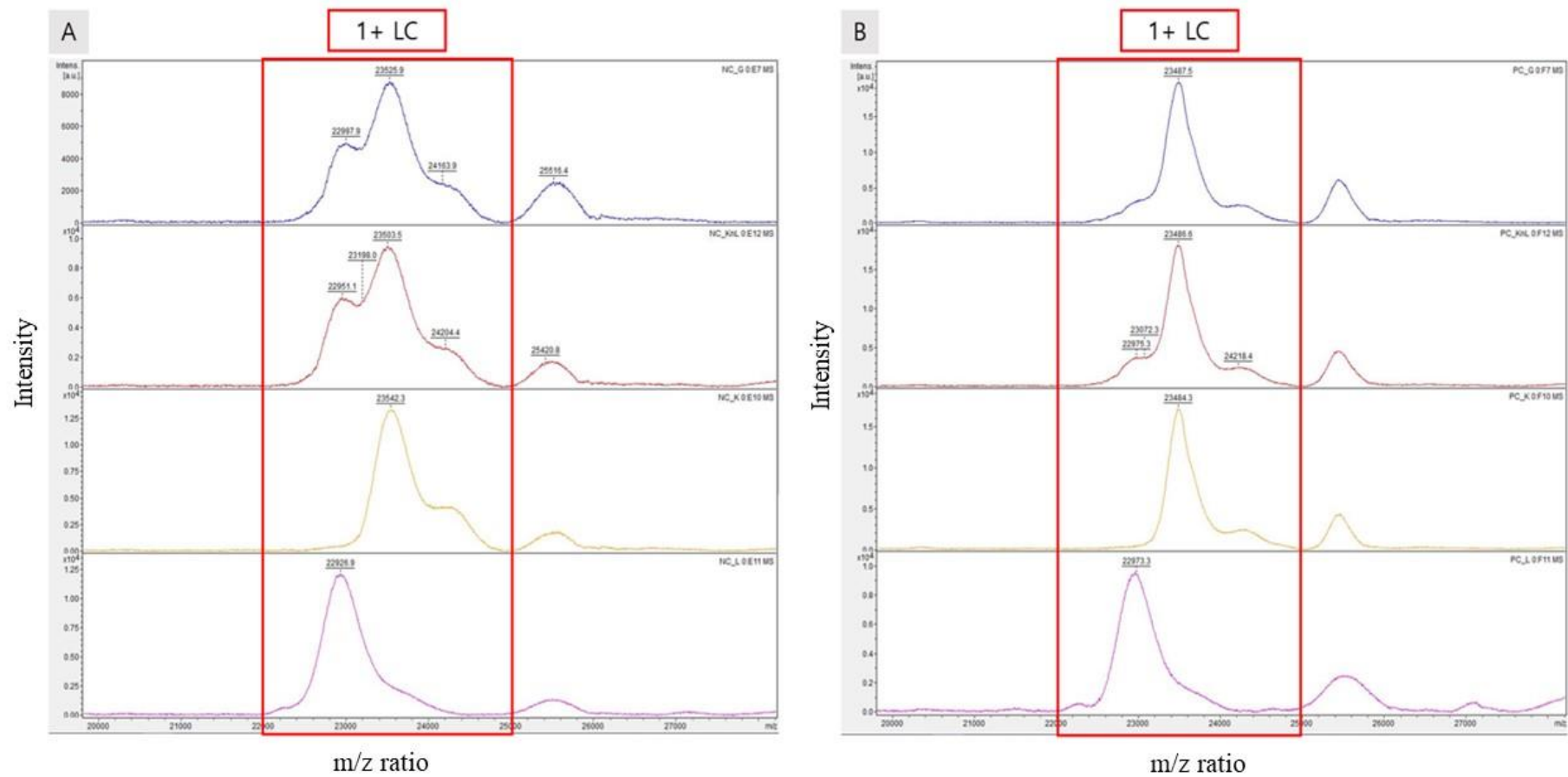
LC charge	Sample	CV, %		
		Melon Kit+C4 ZipTip	MB	NB
1+	Normal	0.09	0.05	0.08
	Abnormal	0.07	0.04	0.06
2+	Normal	0.08	0.07	0.09
	Abnormal	0.03	0.04	0.12

Abbreviations: LC, light chain; MB, magnetic beads; NB, nanobody affinity beads.

Supplemental Data Table S4. Results of five replicates of normal and abnormal samples, tested over 5 days

LC charge	Sample	Statistics	Melon Kit+C4 ZipTip	MB	NB
1+	Normal	Mean	23,340.29	23,319.02	23,342.06
		SD	32.97	24.17	20.69
		CV (%)	0.1%	0.1%	0.1%
	Abnormal	Mean	23,095.72	23,079.06	23,322.4
		SD	23.46	13.6	36.46
		CV (%)	0.1%	0.1%	0.2%
2+	Normal	Mean	11,611.21	11,606.24	11,640.54
		SD	76.95	65.38	110.9
		CV (%)	0.7%	0.6%	1.0%
	Abnormal	Mean	11,497.15	11,495.83	11,634.58
		SD	72.36	66.95	113.24
		CV (%)	0.6%	0.6%	1.0%

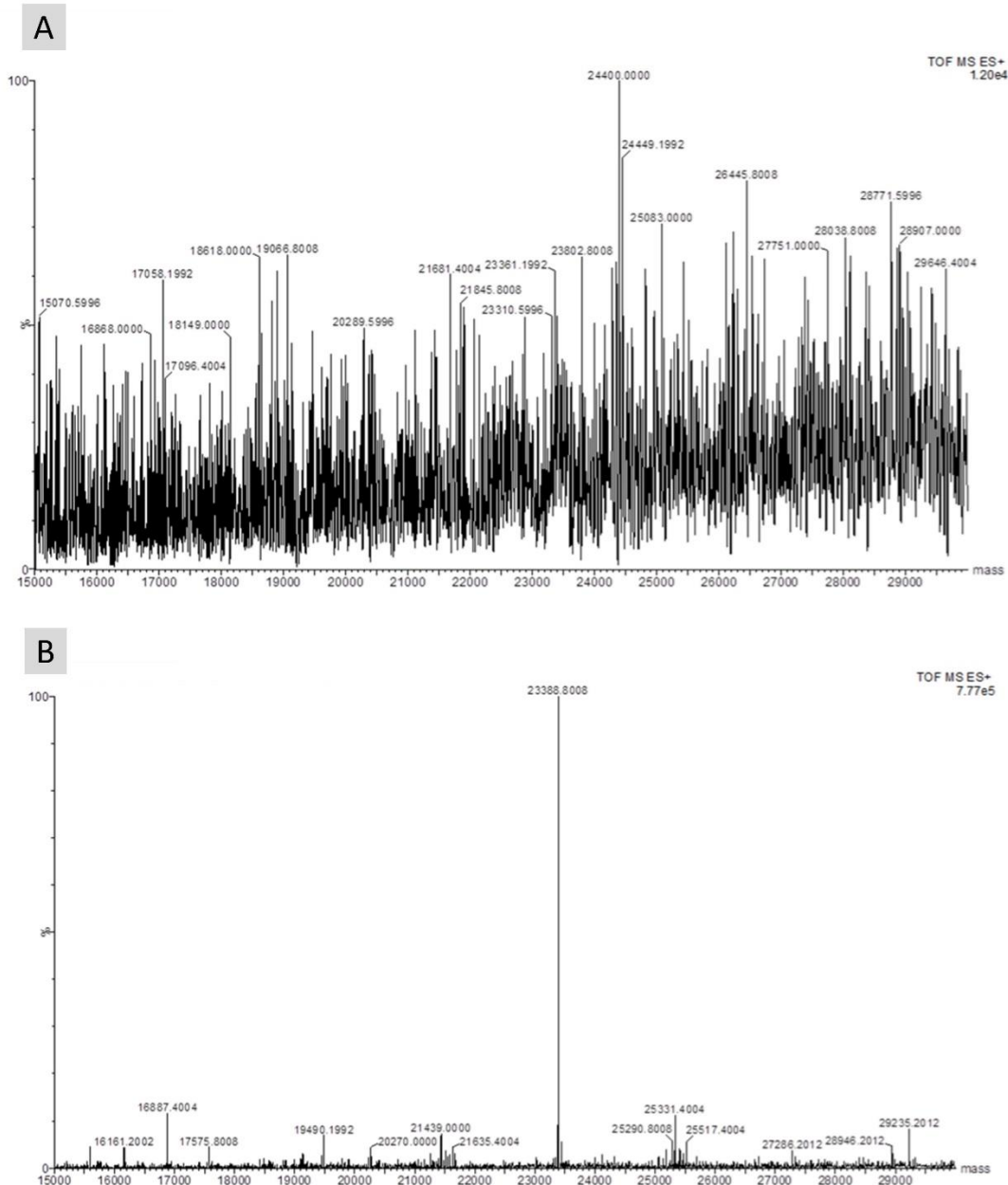
Abbreviations: LC, light chain; MB, magnetic beads; NB, nanobody affinity beads



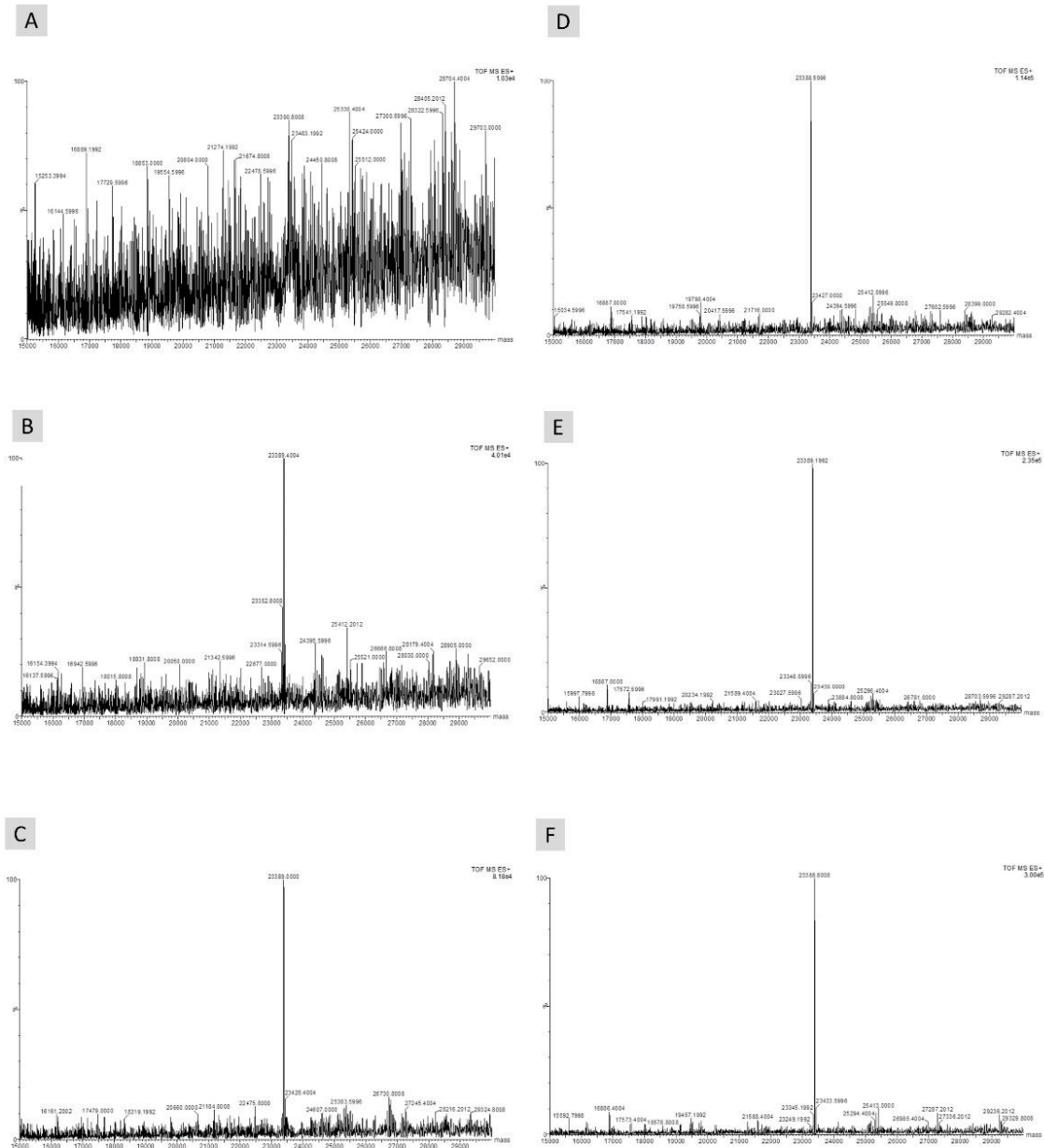
Supplemental Data Fig. S1. Examples of IgG, KnL, kappa, and lambda NB-MALDI-TOF mass spectra of single-charged light chains obtained from normal pooled serum and abnormal serum spiked with daratumumab (0.5 g/dL). (A) With the normal serum, polyclonal peaks were

observed after IgG or KnL purification, whereas single peaks were observed after purification with kappa or lambda beads. (B) With the abnormal (IgG/kappa) serum, monoclonal peaks were observed after IgG or KnL purification, whereas single peaks were observed after purification with kappa or lambda beads. The monoclonality of the IgG/kappa type was confirmed by matching the m/z ratio from a single kappa peak.

Abbreviations: KnL, mixed kappa and lambda; 1+ LC, single-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.



Supplemental Data Fig. S2. Negative and positive controls for NB-LC-ESI-qTOF analysis after reduction with TCEP. (A) A normal pooled serum sample was used as a negative control. (B) A sample spiked with daratumumab (0.5 g/dL) was used as a positive control. Abbreviation: NB-LC-ESI-qTOF, nanobody affinity beads combined with liquid chromatography-electrospray ionization-quadrupole time-of-flight.



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3 **Supplemental Data Fig. S3.** Limit of detection results obtained using NB-LC-ESI-qTOF

4 after IgG purification. (A) Normal pooled serum spiked with daratumumab (0.01 g/dL)

5 showed negative results. (B–F) Serum samples spiked with daratumumab (0.025, 0.05, 0.075,

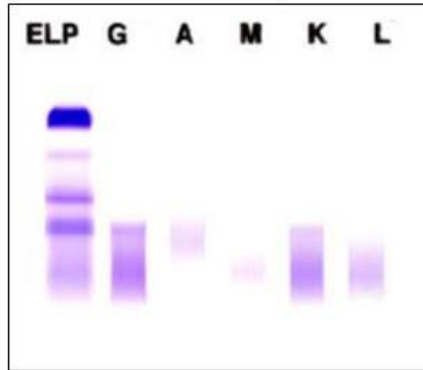
6 0.1, or 0.2 g/dL) showed positive results with monoclonal peaks. The peak intensity increased

7 as the daratumumab concentration increased.

8 Abbreviation: NB-LC-ESI-qTOF, nanobody affinity beads combined with liquid chromatography-

9 electrospray ionization-quadrupole time-of-flight.

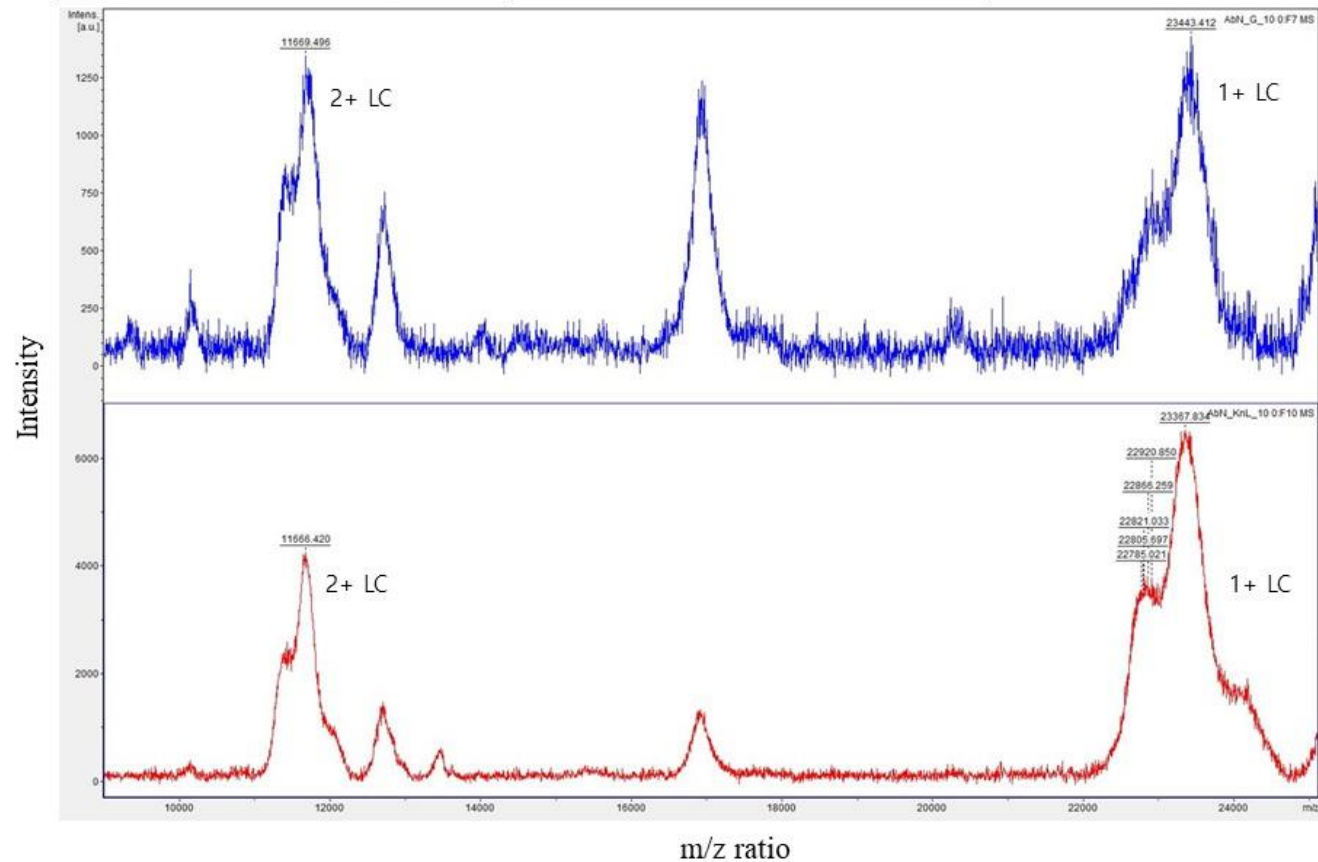
IFE: IgG/kappa



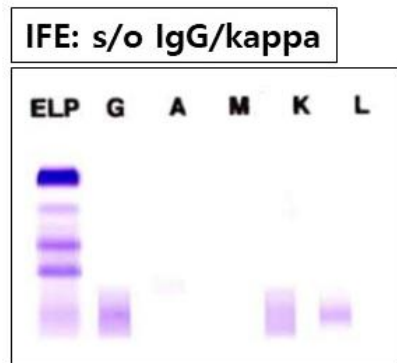
Kappa/lambda ratio

K/L ratio (0.26-1.65)	Kappa (3.3-19.4)	Lambda (5.7-26.3)
1.56	25.4▲	16.3

NB-MALDI-TOF: negative

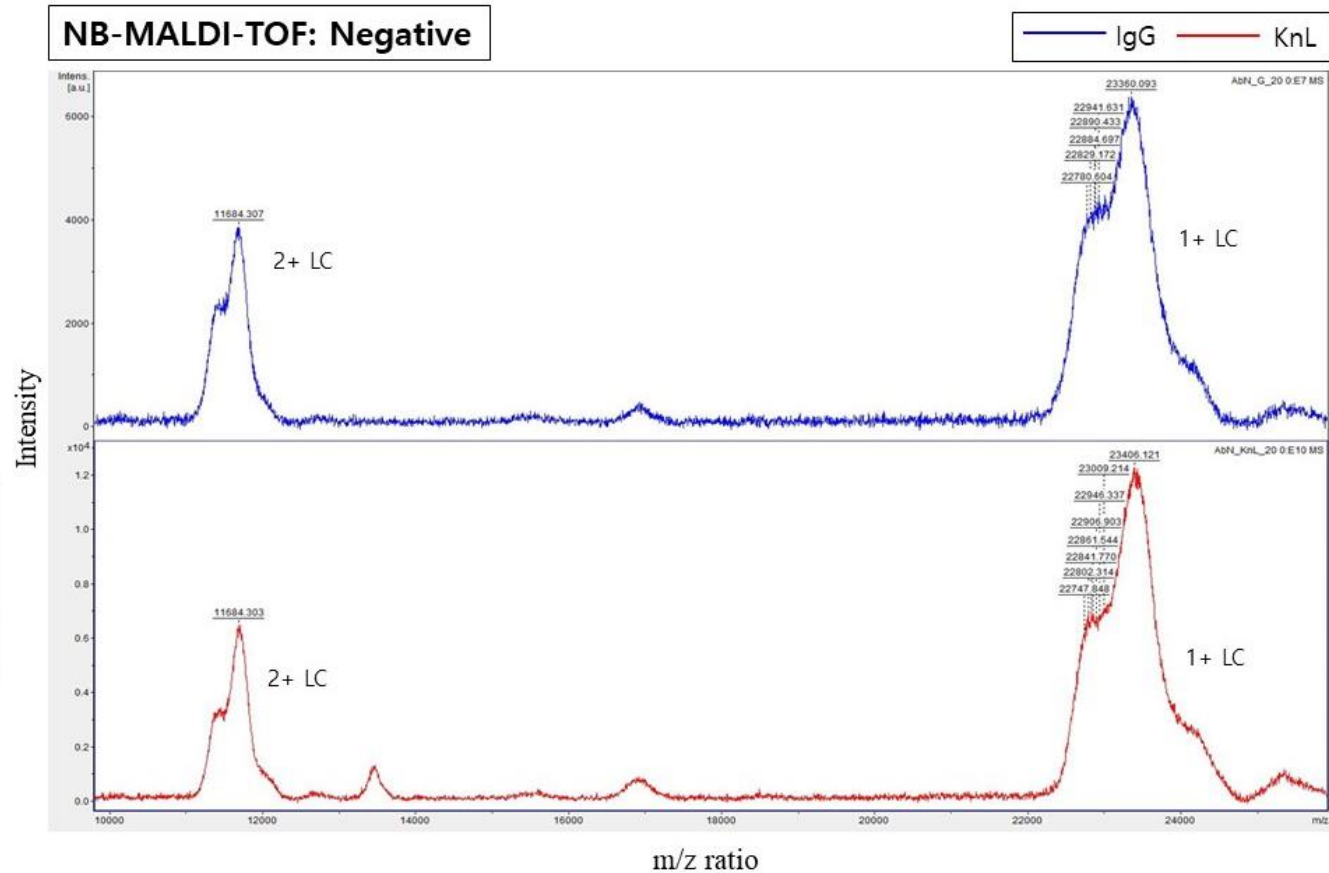


Supplemental Data Fig. S4. First case of a discrepancy between NB-MALDI-TOF and abnormal IFE results. The IgG/kappa subtype identified via IFE (No. 6) showed a negative result via NB-MALDI-TOF. The kappa: lambda ratio was normal with increased kappa chain levels. Abbreviations: IFE, immunofixation electrophoresis; KnL, kappa and lambda mixed beads; 1+ LC, single-charged light chain; 2+ LC, double-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.



Kappa/lambda ratio

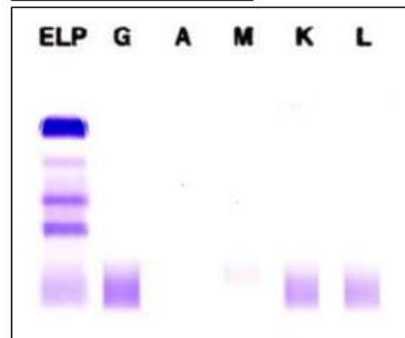
K/L ratio (0.26-1.65)	Kappa (3.3-19.4)	Lambda (5.7-26.3)
34.16 ▲	496.4 ▲	14.5



Supplemental Data Fig. S5. Second case of a discrepancy between NB-MALDI-TOF and abnormal IFE results. A suspected case of an IgG/kappa subtype, as determined via IFE (No. 8), showed a negative result via NB-MALDI-TOF. The kappa: lambda ratio was increased, and the kappa light chain level was markedly increased.

Abbreviations: IFE, immunofixation electrophoresis; KnL, kappa and lambda mixed beads; 1+ LC, single-charged light chain; 2+ LC, double-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.

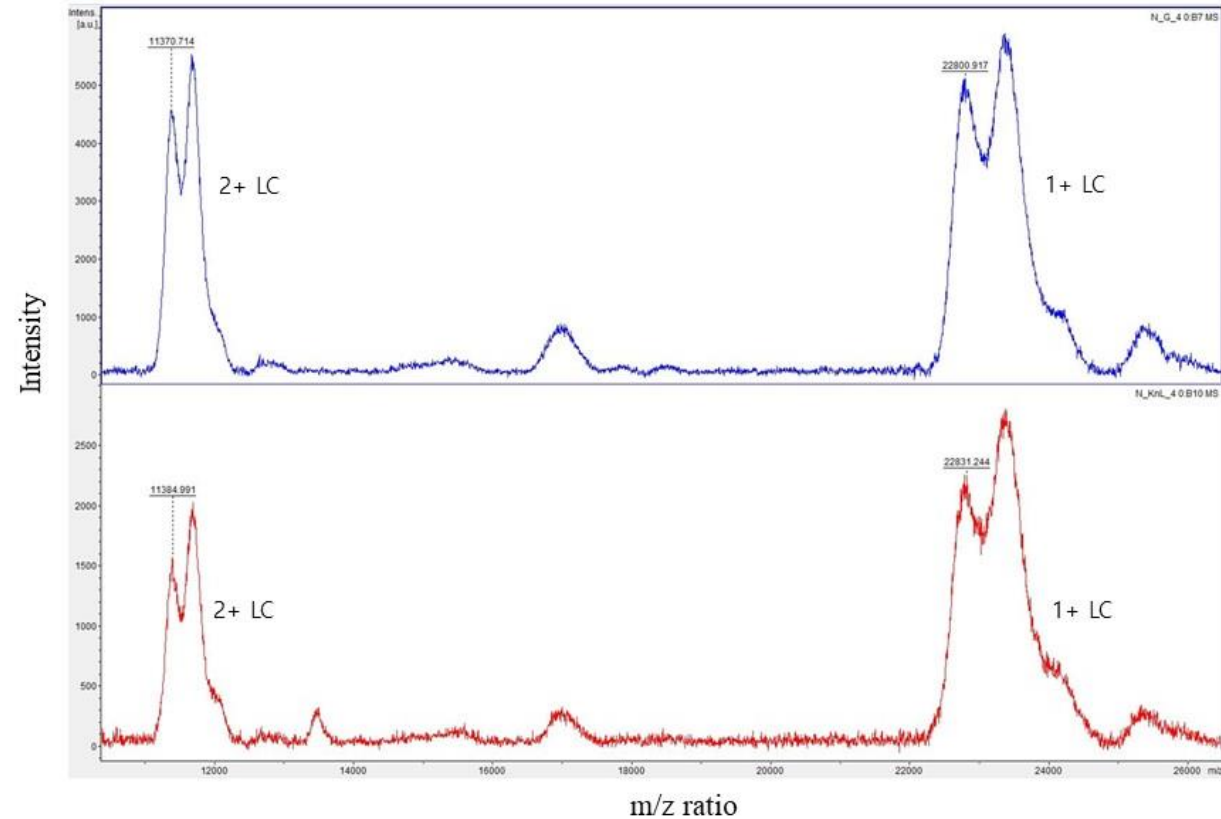
IFE: Negative



Kappa/lambda ratio

K/L ratio (0.26-1.65)	Kappa (3.3-19.4)	Lambda (5.7-26.3)
0.99	6.9	7.0

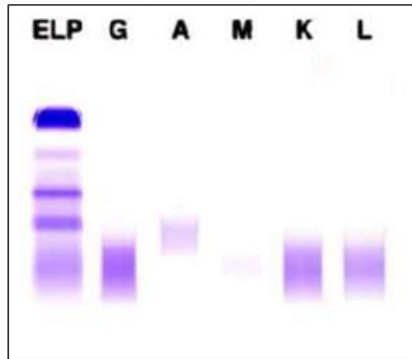
NB-MALDI-TOF: IgG/lambda



Supplemental Data Fig. S6. First case of a discrepancy between NB-MALDI-TOF and normal IFE results. A sample showing a negative result via IFE (No. 3) was positive for IgG/lambda via NB-MALDI-TOF. The kappa: lambda ratio was normal with normal kappa and lambda chain levels.

Abbreviations: IFE, immunofixation electrophoresis; KnL, kappa and lambda mixed beads; 1+ LC, single-charged light chain; 2+ LC, double-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.

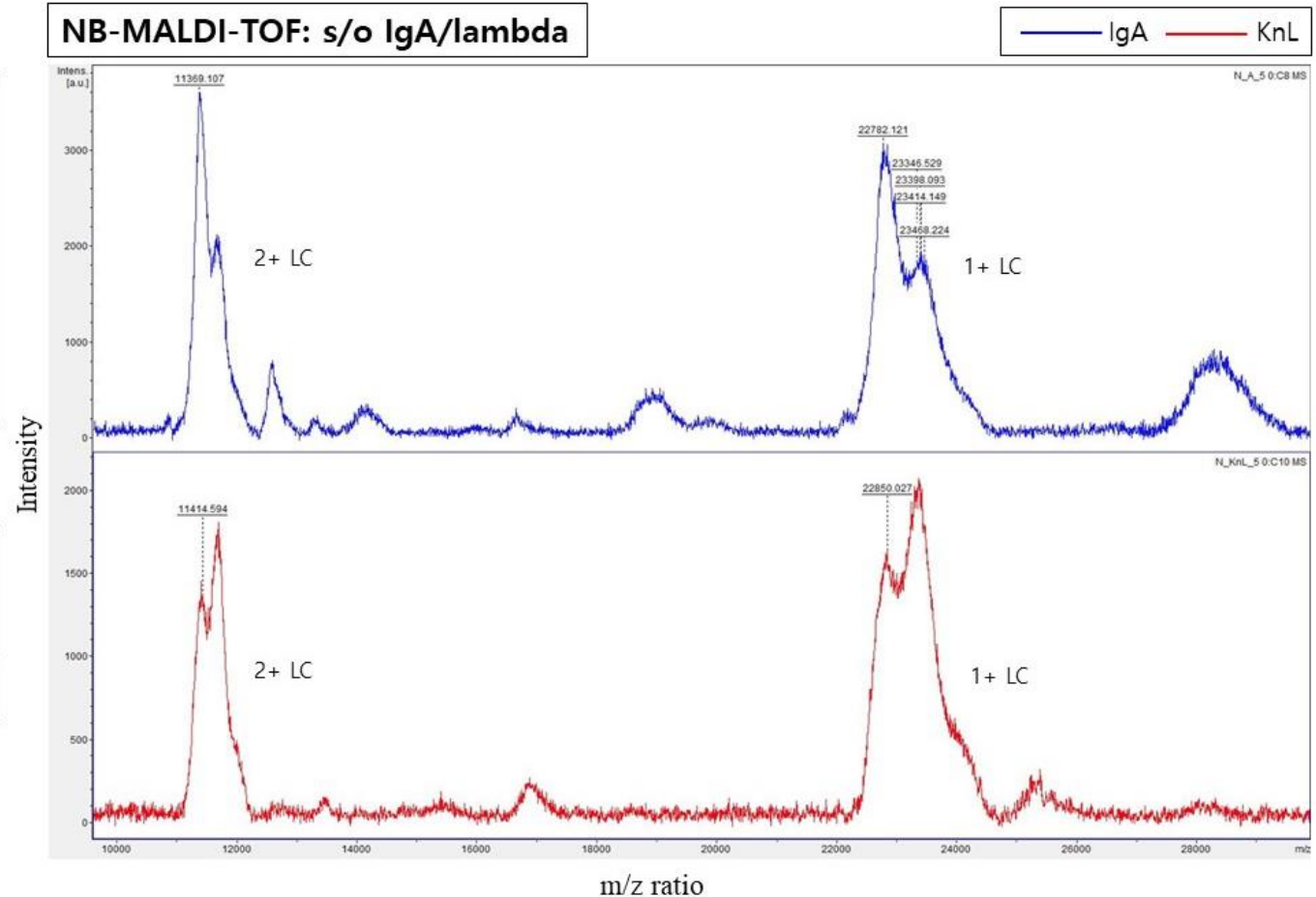
IFE: Negative



Kappa/lambda ratio

K/L ratio (0.26-1.65)	Kappa (3.3-19.4)	Lambda (5.7-26.3)
0.68	15.1	22.3

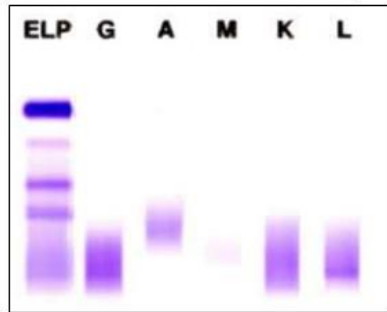
NB-MALDI-TOF: s/o IgA/lambda



Supplemental Data Fig. S7. Second case of a discrepancy between NB-MALDI-TOF and normal IFE results. A sample showing a negative result determined via IFE (No. 4) was suspected of the IgA/lambda subtype based on the NB-MALDI-TOF data. The kappa: lambda ratio was normal with normal kappa and lambda chain levels.

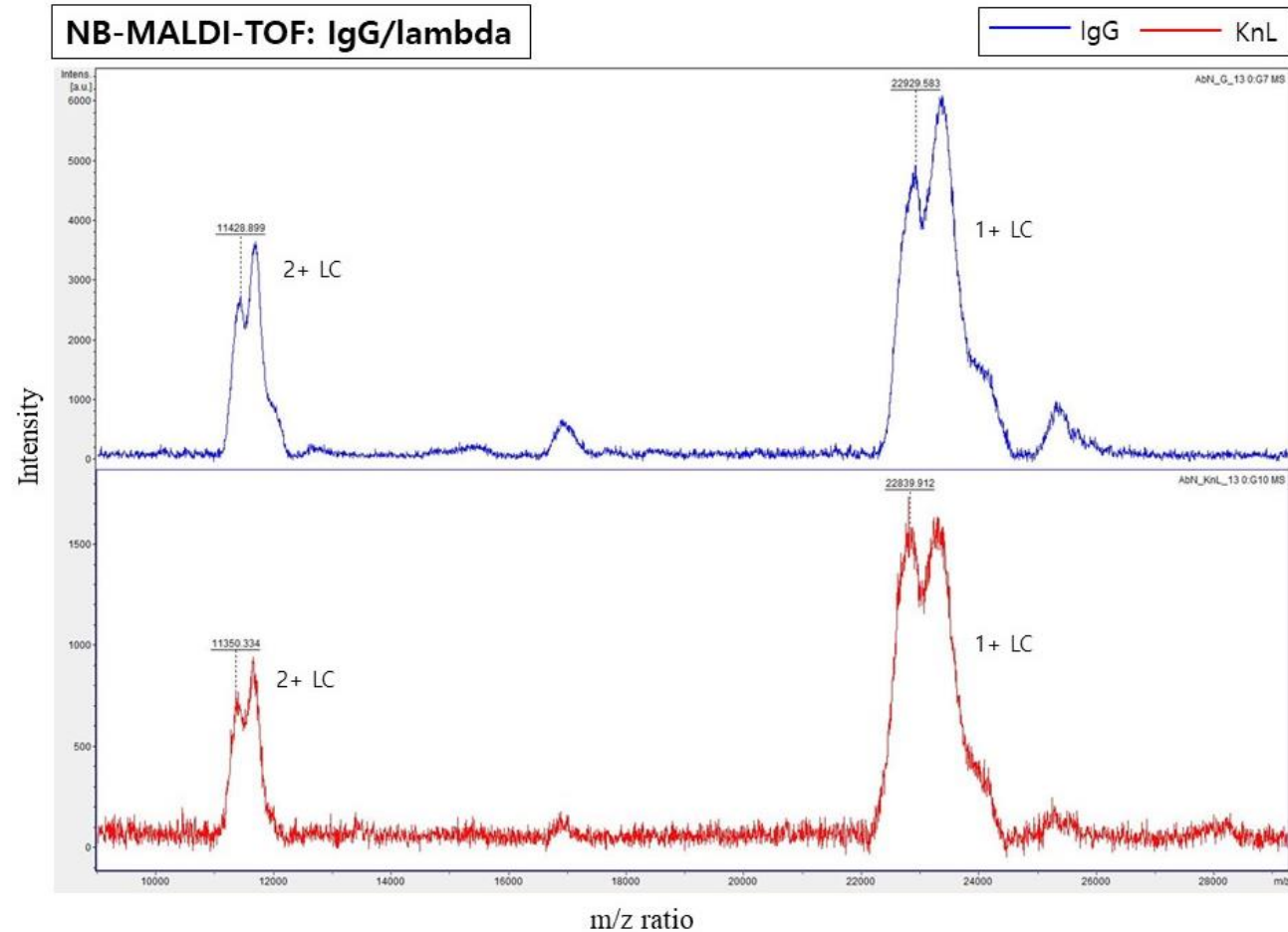
Abbreviations: IFE, immunofixation electrophoresis; KnL, kappa and lambda mixed beads; 1+ LC, single-charged light chain; 2+ LC, double-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.

IFE: s/o IgG/lambda



Kappa/lambda ratio

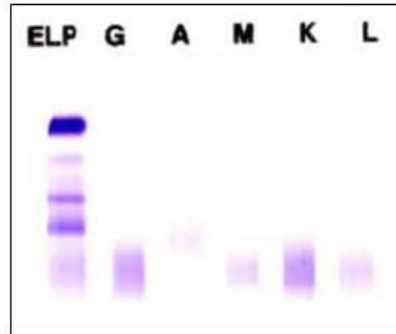
K/L ratio (0.26-1.65)	Kappa (3.3-19.4)	Lambda (5.7-26.3)
1.28	40.5▲	31.6▲



Supplemental Data Fig. S8. First case of a correlation between NB-MALDI-TOF and abnormal IFE results. The IgG/lambda subtype was suspected based on the IFE results (No. 9), and peaks indicating the IgG/lambda subtype were found via NB-MALDI-TOF. The kappa: lambda ratio was normal with increased kappa and lambda chain levels.

Abbreviations: IFE, immunofixation electrophoresis; KnL, kappa and lambda mixed beads; 1+ LC, single-charged light chain; 2+ LC, double-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.

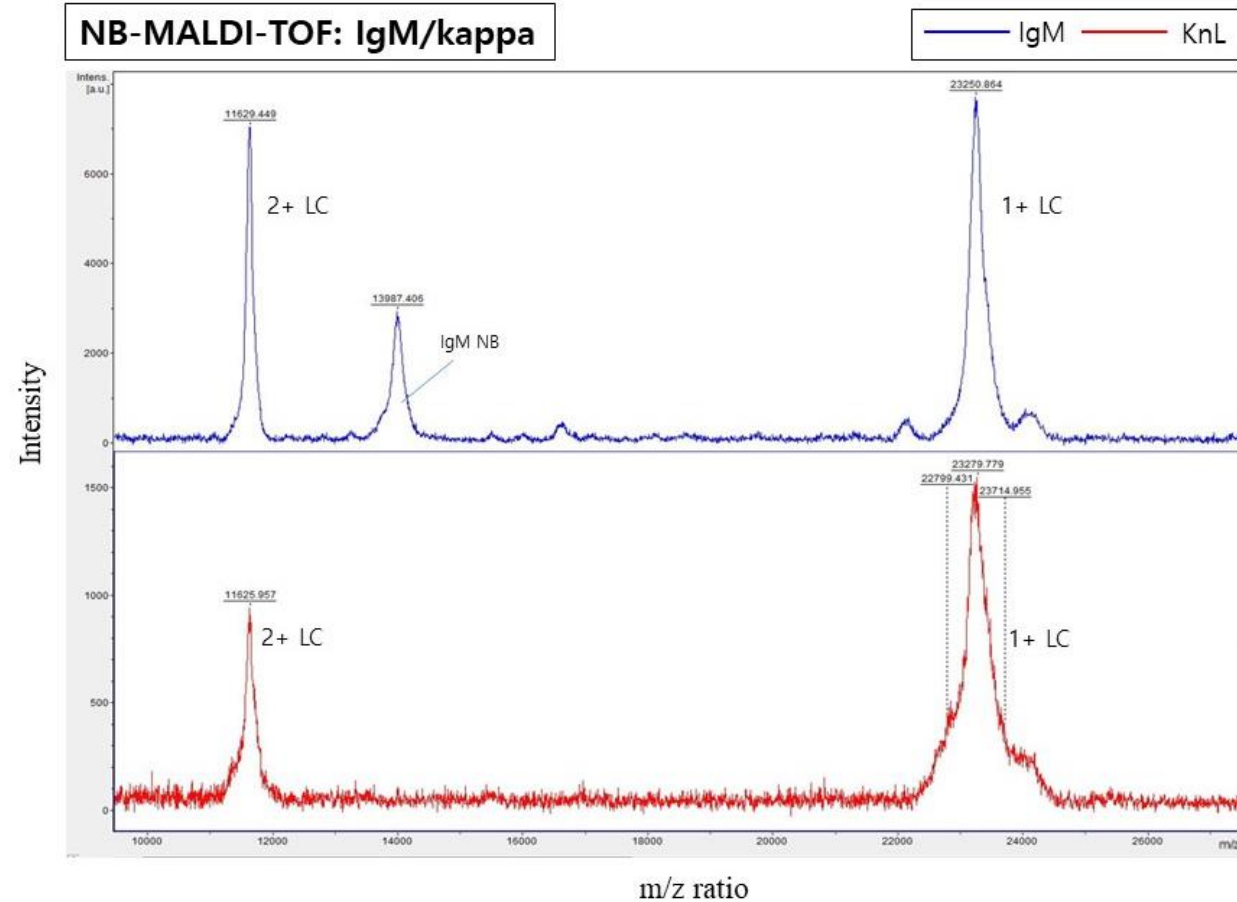
IFE: s/o IgM/kappa



Kappa/lambda ratio

K/L ratio (0.26-1.65)	Kappa (3.3-19.4)	Lambda (5.7-26.3)
1.41	21.2▲	15.1

NB-MALDI-TOF: IgM/kappa



Supplemental Data Fig. S9. Second case of a correlation between NB-MALDI-TOF and abnormal IFE results. The IgM/kappa subtype was suspected based on the IFE results (No. 22) with a dim band, and IgM/kappa peaks were found via NB-MALDI-TOF. The kappa: lambda ratio was normal with increased kappa chain levels.

Abbreviations: IFE, immunofixation electrophoresis; KnL, kappa and lambda mixed beads; 1+ LC, single-charged light chain; 2+ LC, double-charged light chain; NB-MALDI-TOF, nanobody affinity beads combined with matrix-assisted laser desorption/ionization time-of-flight.