

Electronic Supplementary Material (ESI)

Site-specific functionalization with amino, guanidinium, and imidazolyl groups  
enabling activation of 10-23 DNAzyme

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### *T<sub>m</sub> measurement*

Equal molar DNAzyme and substrate D19 were mixed in the reaction buffer (50 mM Tris-HCl, pH 7.5, 2 mM Mg<sup>2+</sup>). On a Cary Bio100, the solution was heated at 85 °C for 10 min, followed by cooling at a rate of 1 °C/min, and the UV absorbance at 260 nm was recorded simultaneously. The A<sub>260</sub> versus temperature curve was derivatized to determine the T<sub>m</sub> values.

### *CD spectra*

Above solutions of DNAzyme-substrate in the reaction buffer (50 mM Tris-HCl, pH 7.5, 2 mM Mg<sup>2+</sup>) were used for CD spectra, on a MOS-450 spectropolarimeter (Biologic, France). At r.t., a sample in a 1 cm path length cuvette was scanned from 200 nm to 350 nm, and three scans were averaged and smoothed with a Savitzky–Golay filter.

Table S1 characterization of DNAzymes with ESI-MS

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DNAzyme	Sequence	MW (calc)	MW (found)
DZ-A5-3	5'-d(tgc tct cca GGC T3G CTA CAA CGA cct gca cct)-3'	10093.6	10094.3
DZ-A9-3	5'-d(tgc tct cca GGC TAG CT3 CAA CGA cct gca cct)-3'	10093.6	10094.6
DZ-A11-3	5'-d(tgc tct cca GGC TAG CTA C3A CGA cct gca cct)-3'	10093.6	10094.2
DZ-A12-3	5'-d(tgc tct cca GGC TAG CTA CA3 CGA cct gca cct)-3'	10093.6	10094.0
DZ-A15-3	5'-d(tgc tct cca GGC TAG CTA CAA CG3 cct gca cct)-3'	10093.6	10094.1
DZ-A0-3	5'-d(tgc tct cc3 GGC TAG CTA CAA CGA cct gca cct)-3'	10093.6	10094.0
DZ-A5-4	5'-d(tgc tct cca GGC T4G CTA CAA CGA cct gca cct)-3'	10123.6	10123.8
DZ-A9-4	5'-d(tgc tct cca GGC TAG CT4 CAA CGA cct gca cct)-3'	10123.6	10122.7
DZ-A11-4	5'-d(tgc tct cca GGC TAG CTA C4A CGA cct gca cct)-3'	10123.6	10123.5
DZ-A 12-4	5'-d(tgc tct cca GGC TAG CTA CA4 CGA cct gca cct)-3'	10123.6	10126.6
DZ-A15-4	5'-d(tgc tct cca GGC TAG CTA CAA CG4 cct gca cct)-3'	10123.6	10123.2
DZ-A0-4	5'-d(tgc tct cc4 GGC TAG CTA CAA CGA cct gca cct)-3'	10123.6	10123.4
DZ-A5-5	5'-d(tgc tct cca GGC T5G CTA CAA CGA cct gca cct)-3'	10093.0	10095.0
DZ-A9-5	5'-d(tgc tct cca GGC TAG CT5 CAA CGA cct gca cct)-3'	10093.0	10092.8
DZ-A11-5	5'-d(tgc tct cca GGC TAG CTA C5A CGA cct gca cct)-3'	10093.0	10091.7
DZ-A 12-5	5'-d(tgc tct cca GGC TAG CTA CA5 CGA cct gca cct)-3'	10093.0	10097.6
DZ-A15-5	5'-d(tgc tct cca GGC TAG CTA CAA CG5 cct gca cct)-3'	10093.0	10091.4

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Table S1 (continued)

DNAzyme	Sequence	MW (calc)	MW (found)
DZ-A0-5	5'-d(tgc tct cc <b>5</b> GGC TAG CTA CAA CGA cct gca cct)-3'	10093.0	10091.7
DZ-A5-6	5'-d(tgc tct cca GGC T <b>6</b> G CTA CAA CGA cct gca cct)-3'	10102.4	10102.4
DZ-A9-6	5'-d(tgc tct cca GGC TAG CT <b>6</b> CAA CGA cct gca cct)-3'	10102.4	10102.4
DZ-A11-6	5'-d(tgc tct cca GGC TAG CTA C <b>6</b> A CGA cct gca cct)-3'	10102.4	10102.0
DZ-A 12-6	5'-d(tgc tct cca GGC TAG CTA CA <b>6</b> CGA cct gca cct)-3'	10102.4	10102.1
DZ-A15-6	5'-d(tgc tct cca GGC TAG CTA CAA CG <b>6</b> cct gca cct)-3'	10102.4	10102.0
DZ-A0-6	5'-d(tgc tct cc <b>6</b> GGC TAG CTA CAA CGA cct gca cct)-3'	10102.4	10103.1
DZ-A5-7	5'-d(tgc tct cca GGC T <b>7</b> G CTA CAA CGA cct gca cct)-3'	10066.0	10066.6
DZ-A9-7	5'-d(tgc tct cca GGC TAG CT <b>7</b> CAA CGA cct gca cct)-3'	10066.0	10067.3
DZ-A11-7	5'-d(tgc tct cca GGC TAG CTA C <b>7</b> A CGA cct gca cct)-3'	10066.0	10066.3
DZ-A 12-7	5'-d(tgc tct cca GGC TAG CTA CA <b>7</b> CGA cct gca cct)-3'	10066.0	10066.5
DZ-A15-7	5'-d(tgc tct cca GGC TAG CTA CAA CG <b>7</b> cct gca cct)-3'	10066.0	10066.2
DZ-A0-7	5'-d(tgc tct cc <b>7</b> GGC TAG CTA CAA CGA cct gca cct)-3'	10066.0	10066.5
DZ-A5-8	5'-d(tgc tct cca GGC T <b>8</b> G CTA CAA CGA cct gca cct)-3'	10102.7	10103.2
DZ-A9-8	5'-d(tgc tct cca GGC TAG CT <b>8</b> CAA CGA cct gca cct)-3'	10102.7	10102.5
DZ-A11-8	5'-d(tgc tct cca GGC TAG CTA C <b>8</b> A CGA cct gca cct)-3'	10102.7	10104.0
DZ-A12-8	5'-d(tgc tct cca GGC TAG CTA CA <b>8</b> CGA cct gca cct)-3'	10102.7	10102.4
DZ-A15-8	5'-d(tgc tct cca GGC TAG CTA CAA CG <b>8</b> cct gca cct)-3'	10102.7	10102.4
DZ-A0-8	5'-d(tgc tct cc <b>8</b> GGC TAG CTA CAA CGA cct gca cct)-3'	10102.7	10105.1
DZ-A5-9	5'-d(tgc tct cca GGC T <b>9</b> G CTA CAA CGA cct gca cct)-3'	10093.0	10092.2
DZ-A9-9	5'-d(tgc tct cca GGC TAG CT <b>9</b> CAA CGA cct gca cct)-3'	10093.0	10091.8
DZ-A11-9	5'-d(tgc tct cca GGC TAG CTA C <b>9</b> A CGA cct gca cct)-3'	10093.0	10091.2
DZ-A12-9	5'-d(tgc tct cca GGC TAG CTA CA <b>9</b> CGA cct gca cct)-3'	10093.0	10094.6
DZ-A15-9	5'-d(tgc tct cca GGC TAG CTA CAA CG <b>9</b> cct gca cct)-3'	10093.0	10091.4
DZ-A0-9	5'-d(tgc tct cc <b>9</b> GGC TAG CTA CAA CGA cct gca cct)-3'	10093.0	10093.2

Table S2  $T_m$  of modified DNAzymes with its complementary DNA substrate<sup>a</sup>

DNAzyme	$T_m$ (°C)	DNAzyme	$T_m$ (°C)	DNAzyme	$T_m$ (°C)
DZ01	50.2				
DZ-A0-3	51.6	DZ-A0-4	48.7	DZ-A0-5	51.0
DZ-A5-3	51.8	DZ-A5-4	50.6	DZ-A5-5	49.8
DZ-A9-3	52.2	DZ-A9-4	50.9	DZ-A9-5	50.7
DZ-A11-3	51.6	DZ-A11-4	50.0	DZ-A11-5	50.3
DZ-A12-3	52.3	DZ-A12-4	50.2	DZ-A12-5	50.3
DZ-A15-3	52.5	DZ-A15-4	50.3	DZ-A15-5	50.3
DZ-A0-6	48.8	DZ-A0-7	51.1	DZ-A0-8	48.6
DZ-A5-6	49.5	DZ-A5-7	50.4	DZ-A5-8	49.4
DZ-A9-6	49.8	DZ-A9-7	49.1	DZ-A9-8	49.7
DZ-A11-6	50.1	DZ-A11-7	49.2	DZ-A11-8	50.1
DZ-A12-6	50.2	DZ-A12-7	50.1	DZ-A12-8	50.0
DZ-A15-6	50.6	DZ-A15-7	52.3	DZ-A15-8	49.5
DZ-A0-9	49.8	DZ-A5-9	50.8	DZ-A9-9	51.0
DZ-A11-9	50.0	DZ-A12-9	50.9	Z-A15-9	49.7

<sup>a</sup>Measured under the reaction conditions (50 mM Tris-HCl, pH 7.5, 2 mM Mg<sup>2+</sup>).

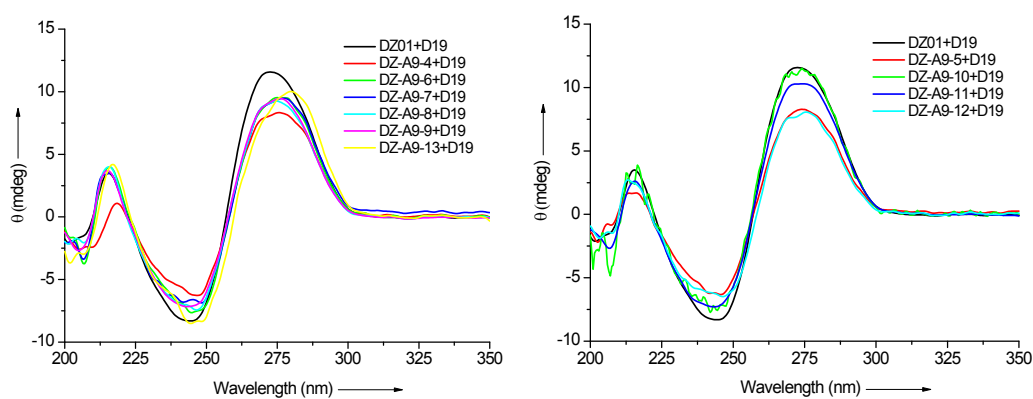
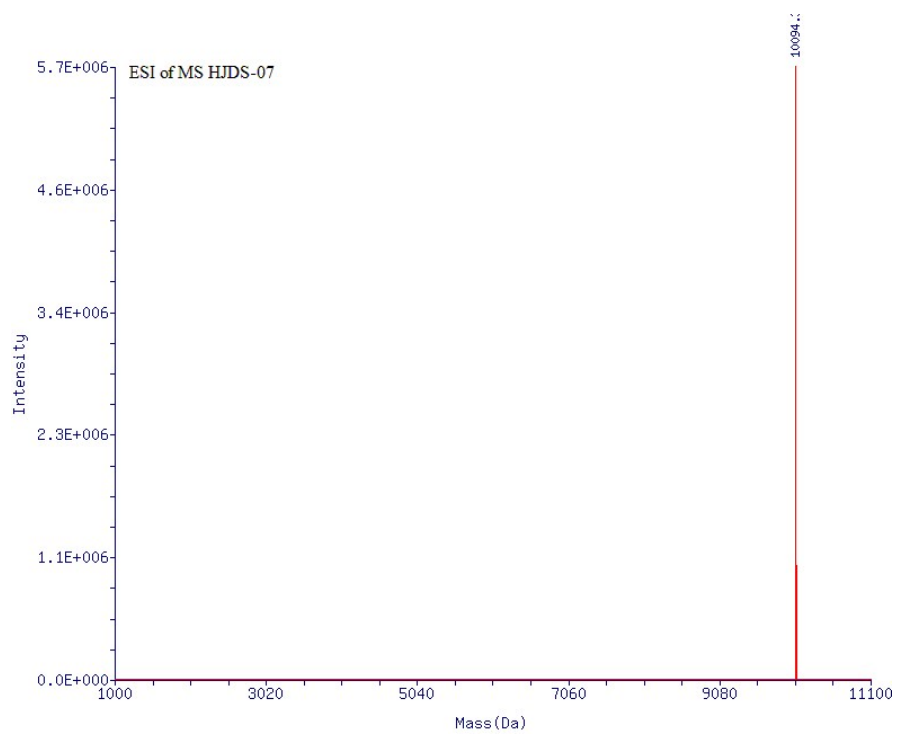
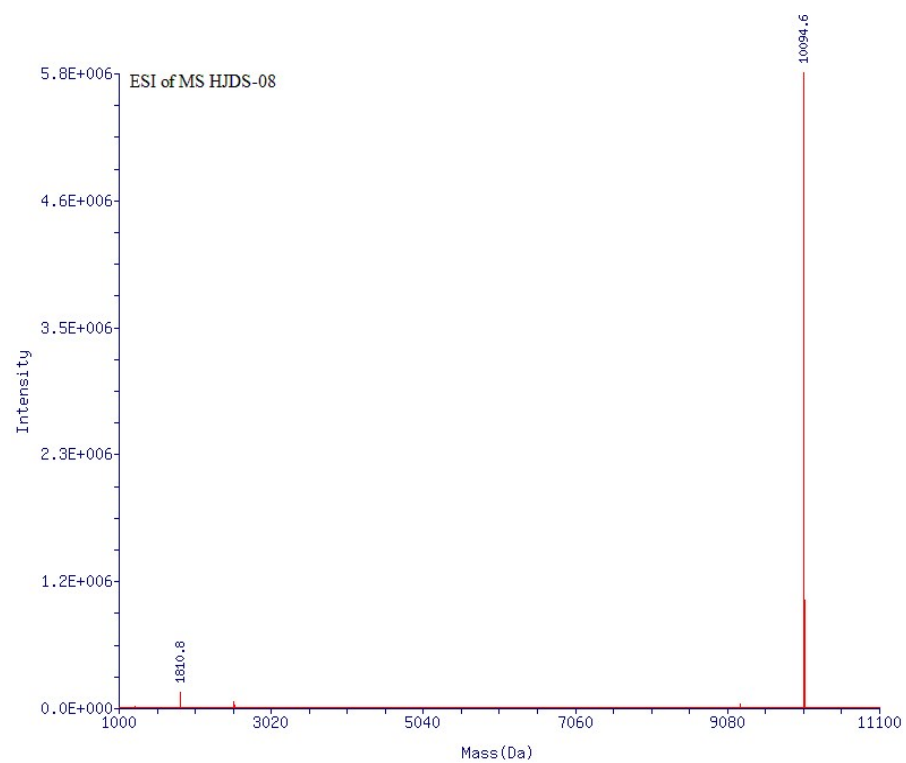


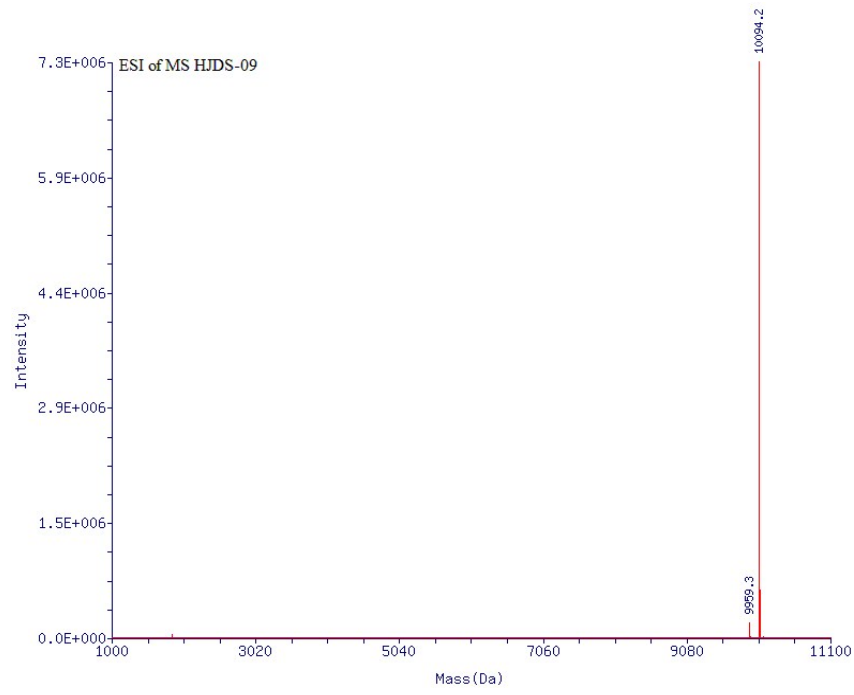
Fig. S1 CD spectra of DNAzymes with the full-DNA substrate under the reaction conditions (50 mM Tris-HCl, pH 7.5, 2 mM Mg<sup>2+</sup>)



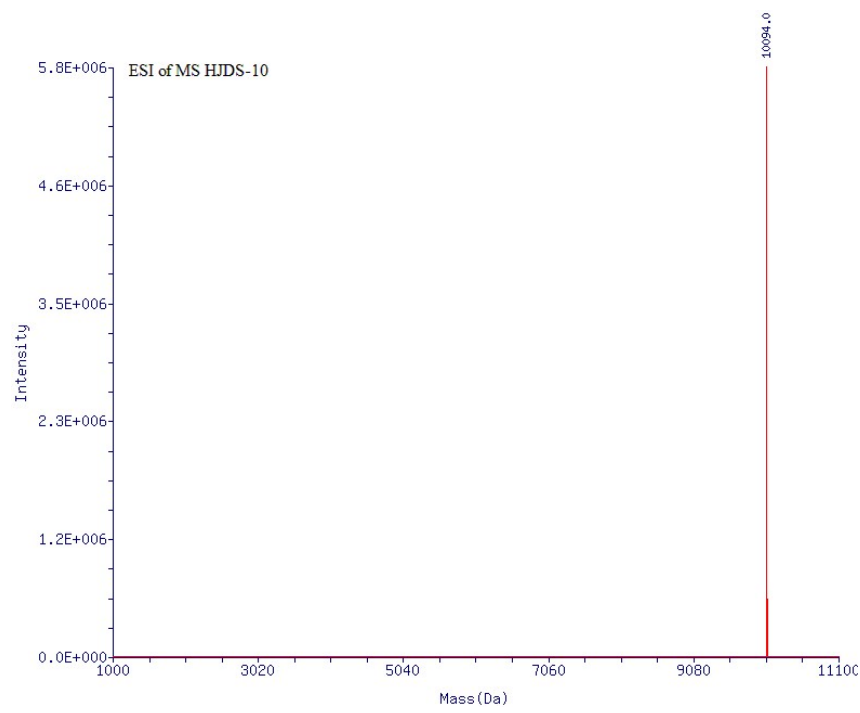
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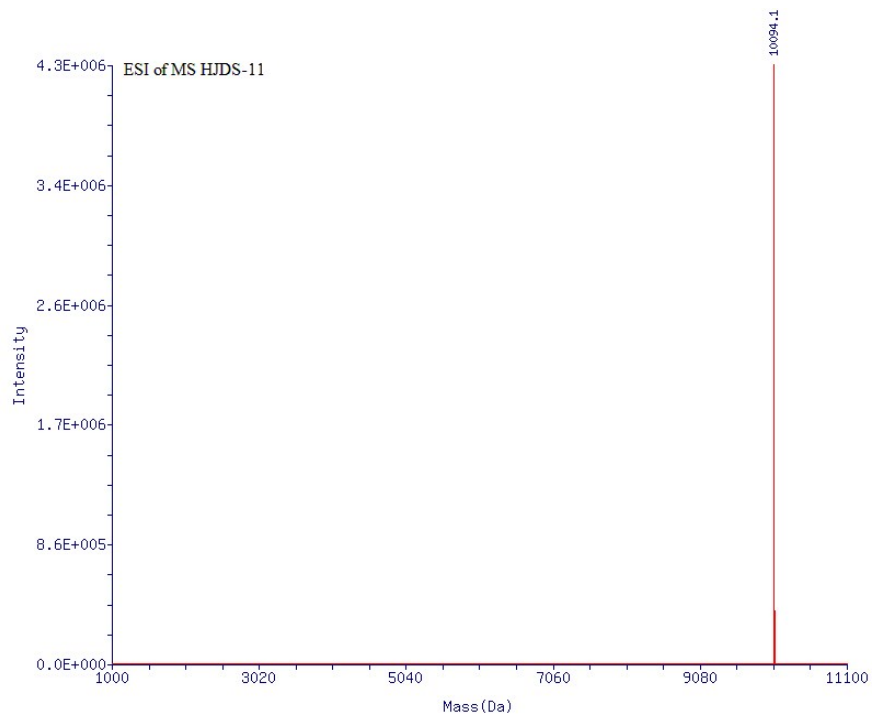
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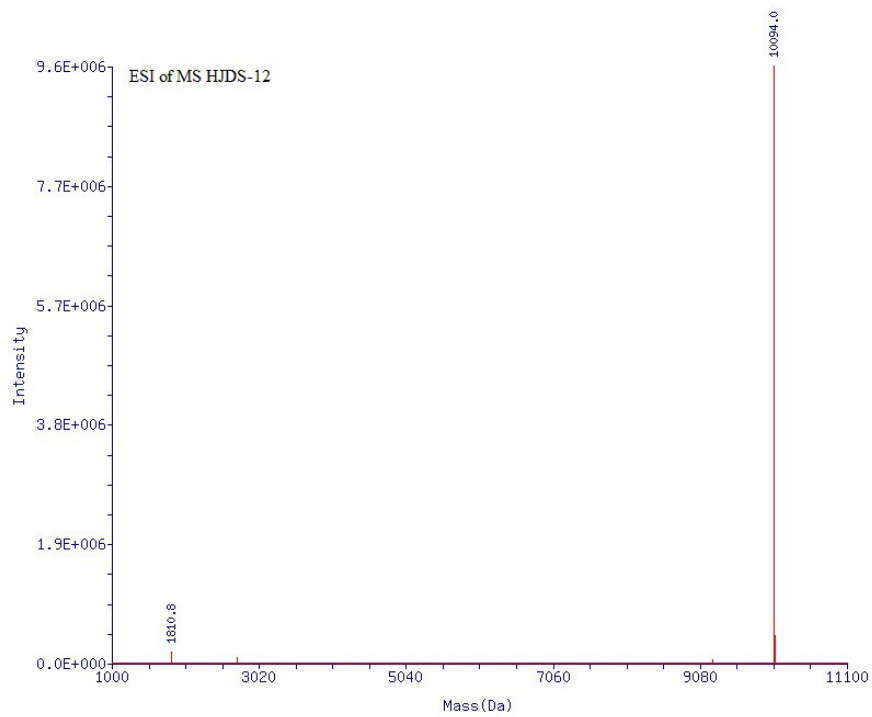
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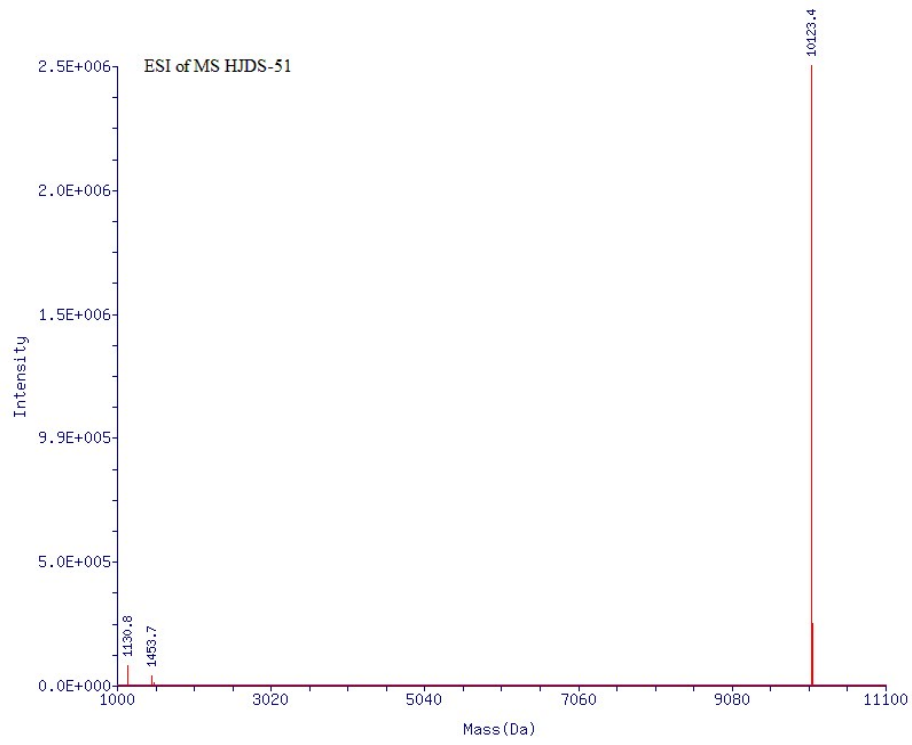
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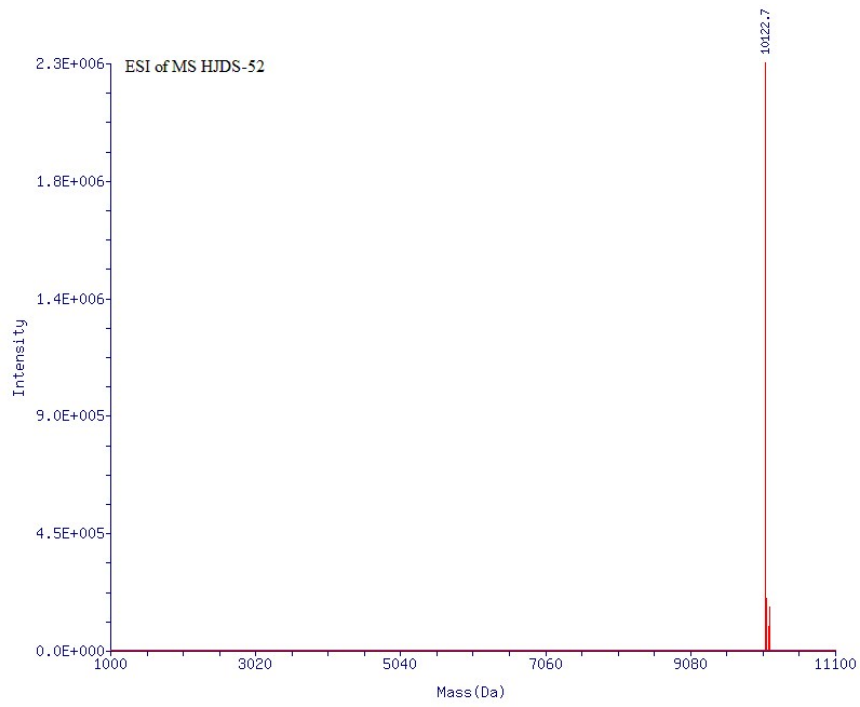
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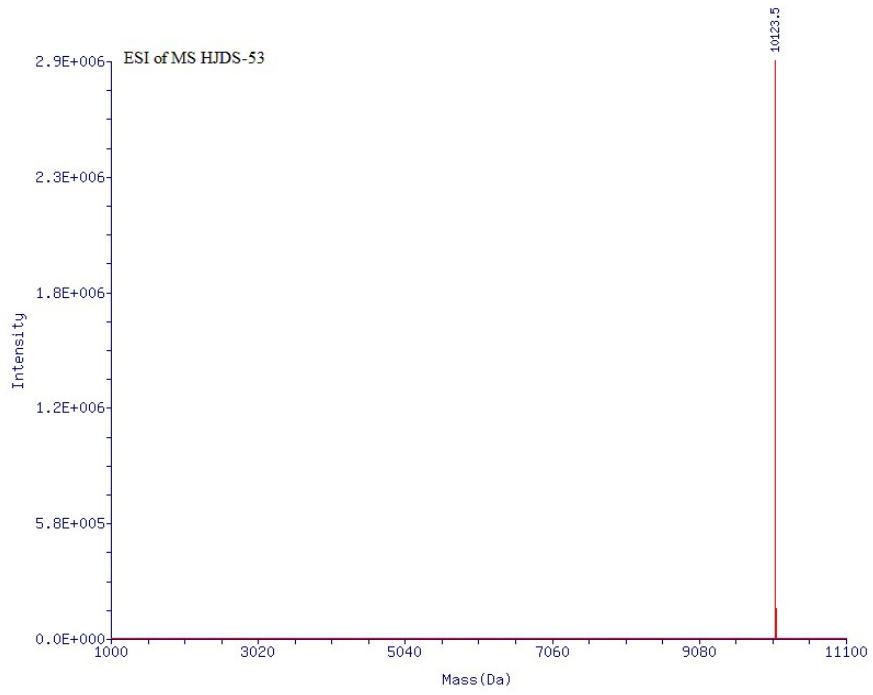


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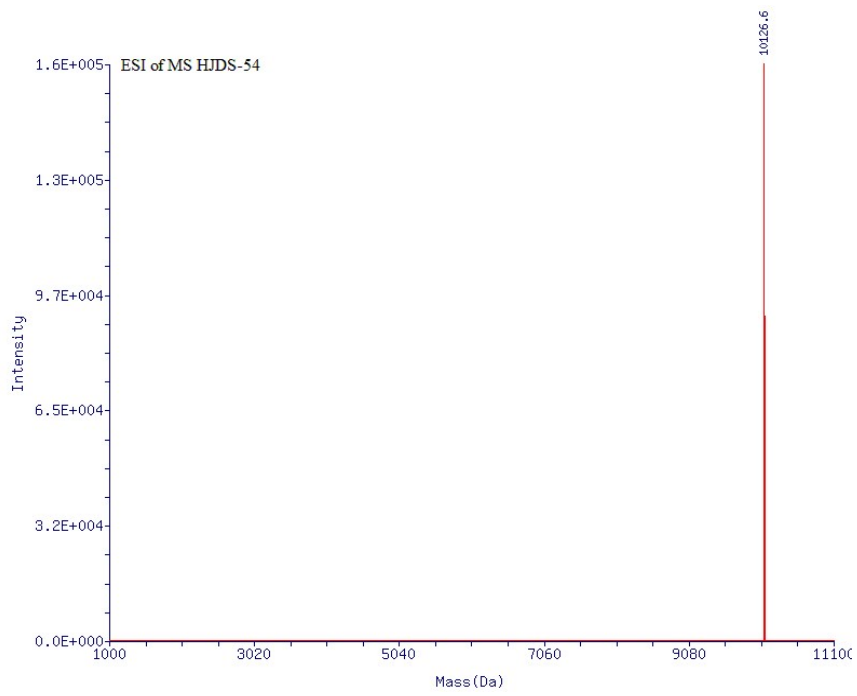


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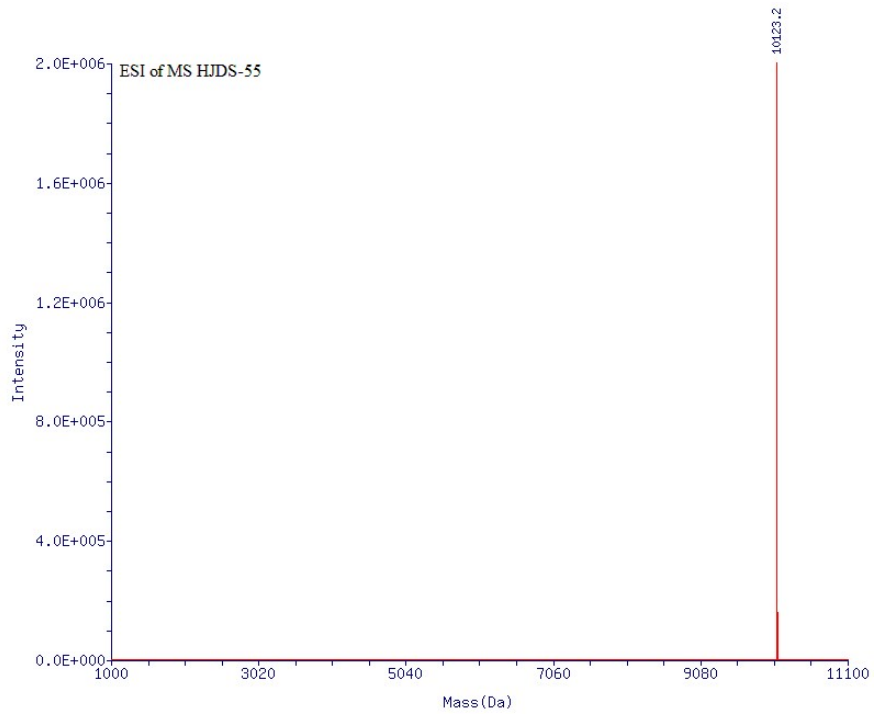




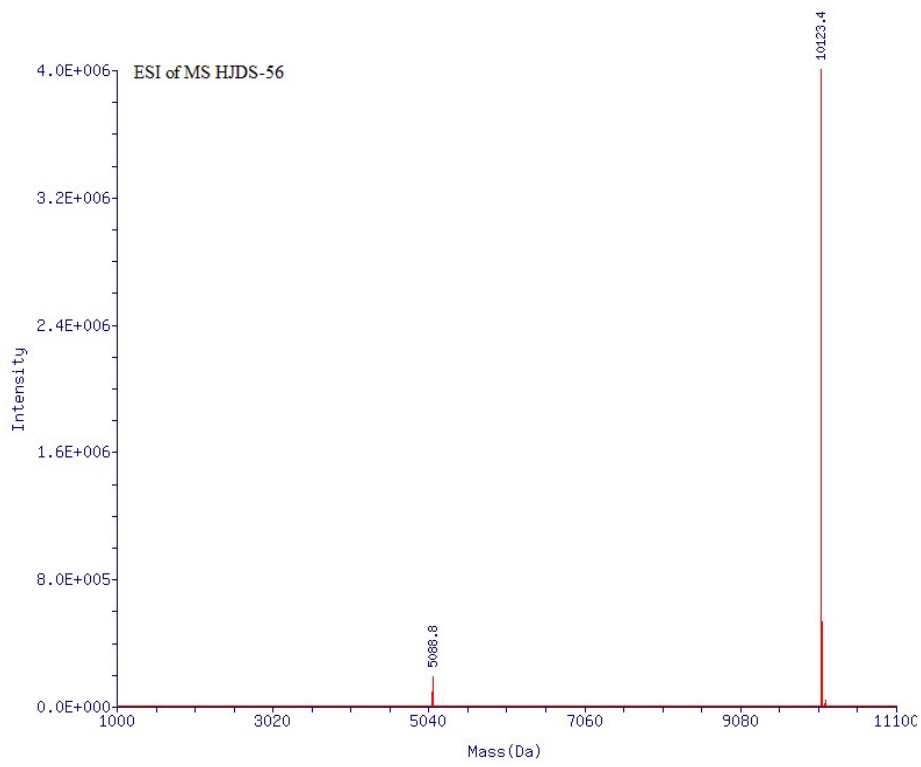
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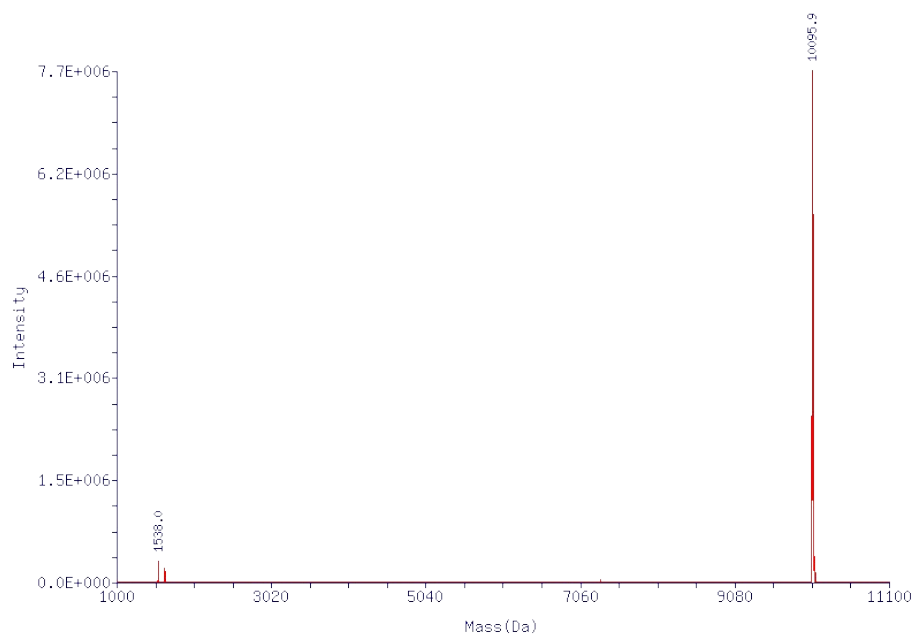
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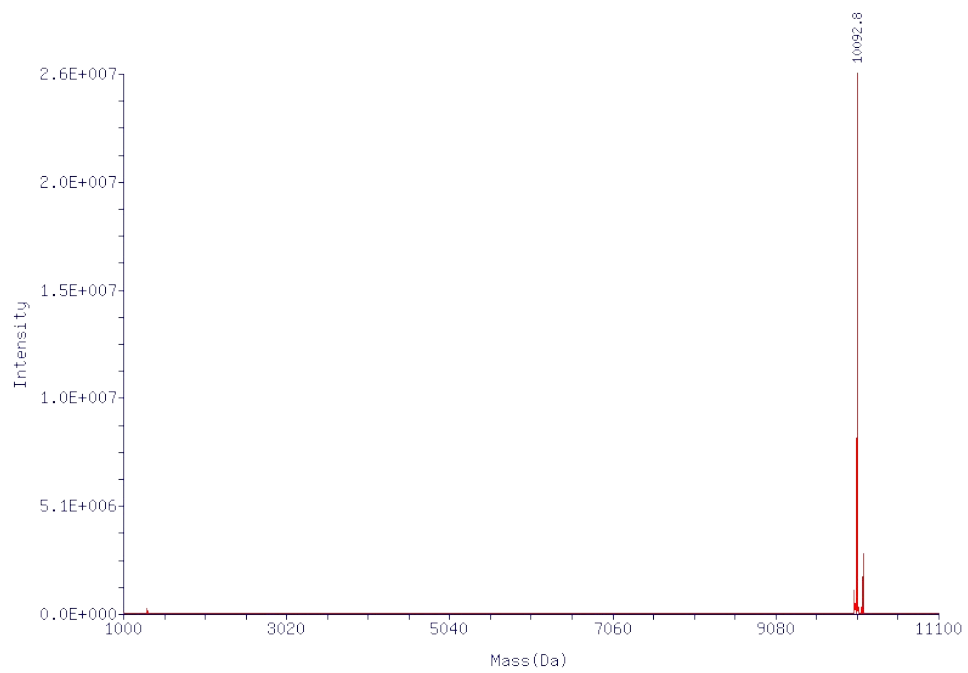
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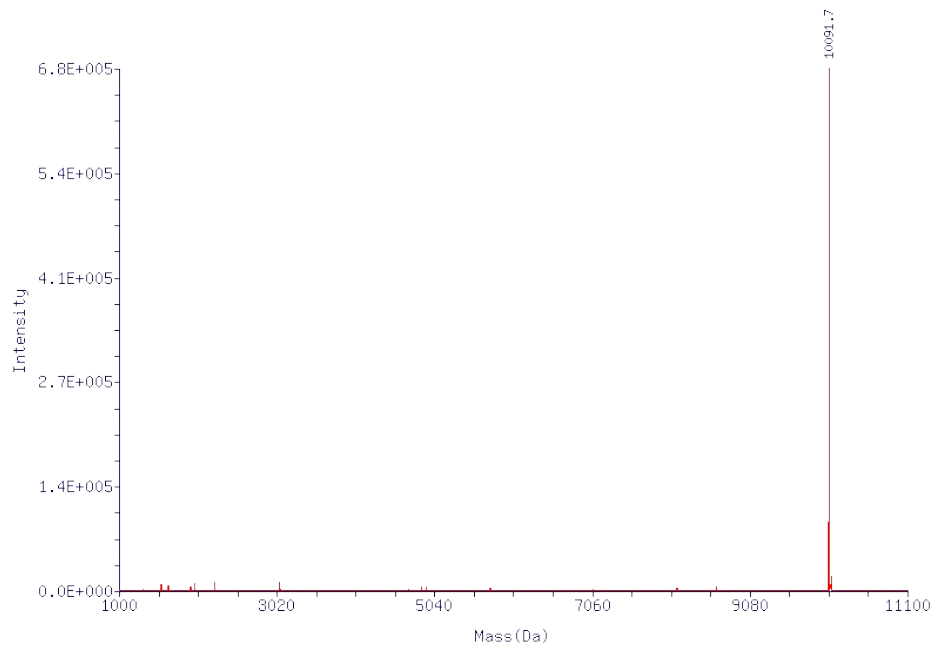


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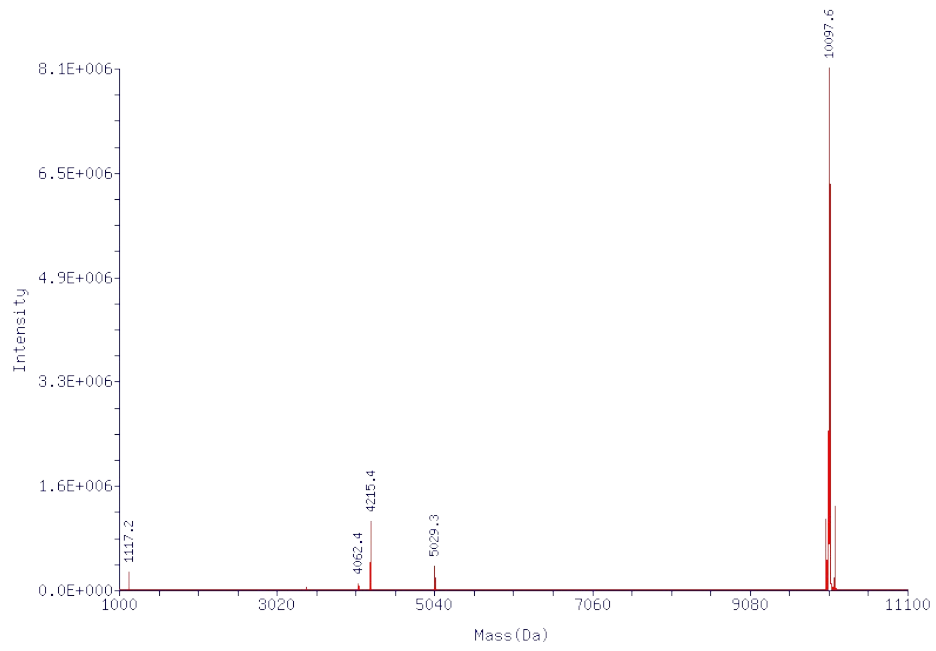
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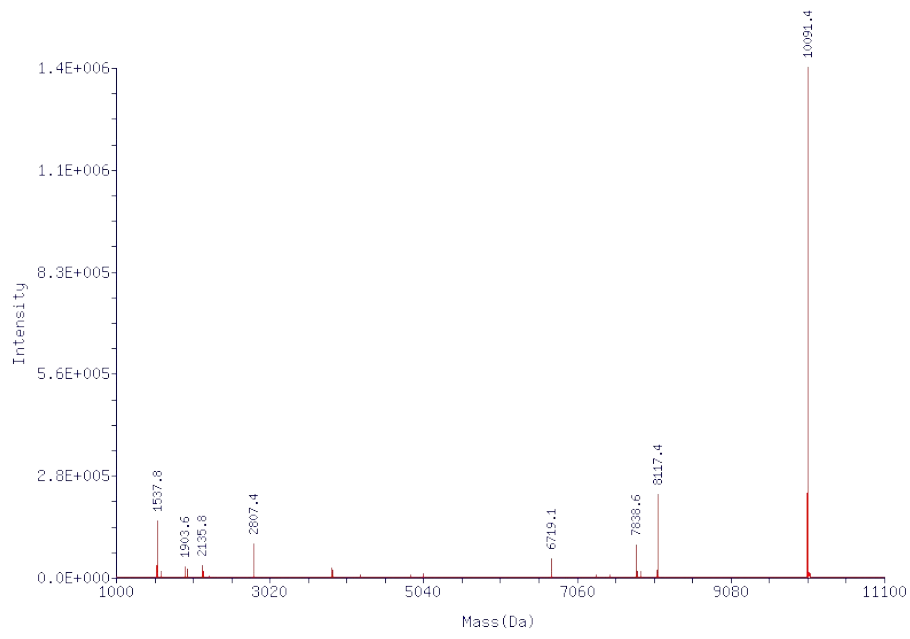
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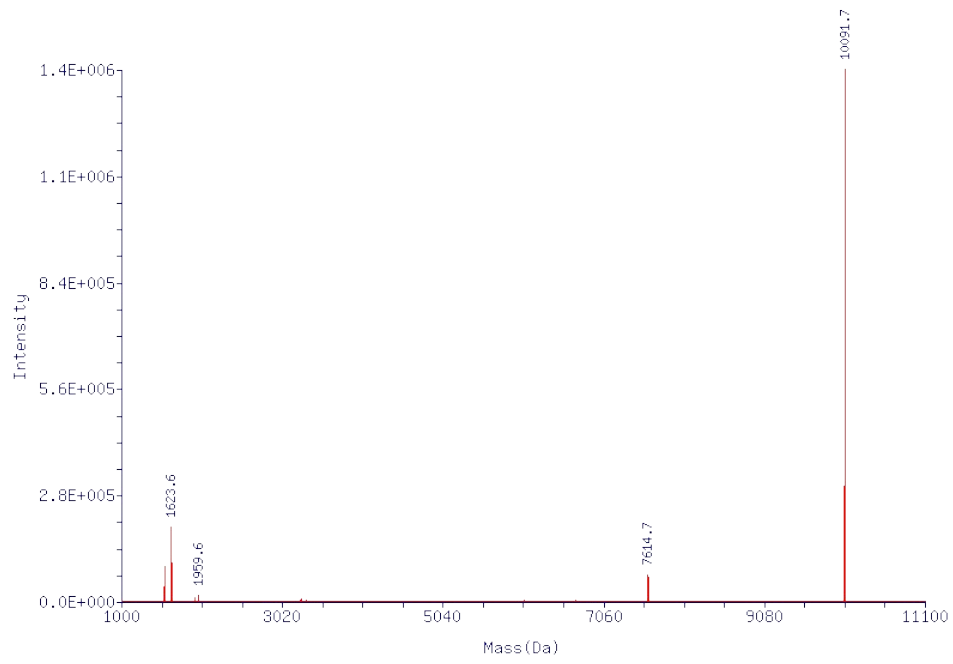


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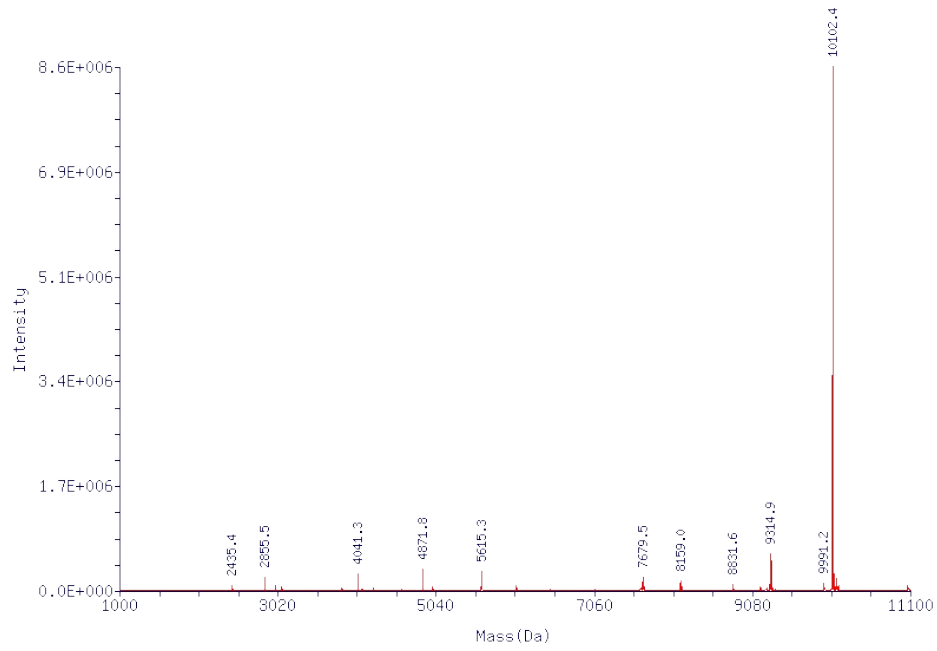
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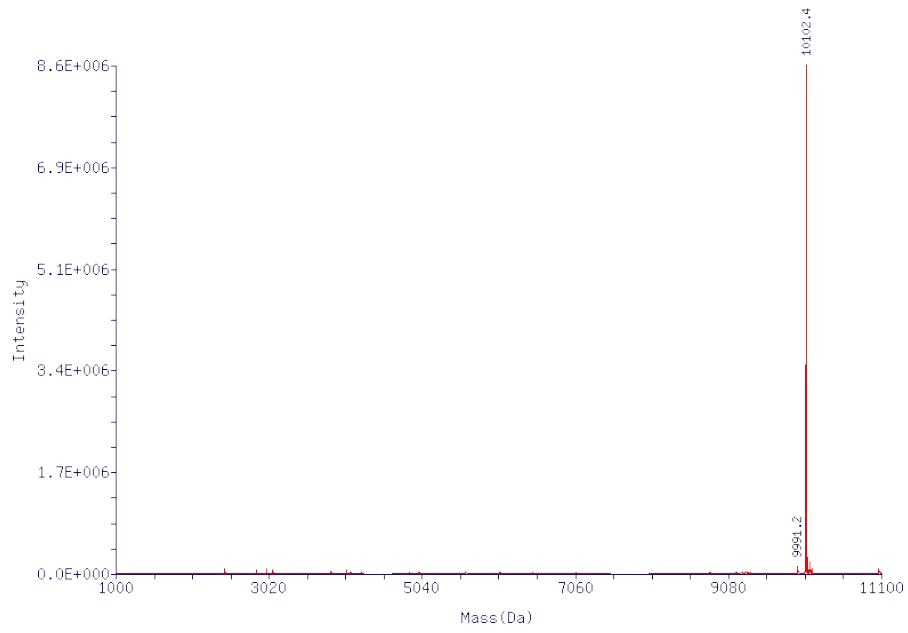
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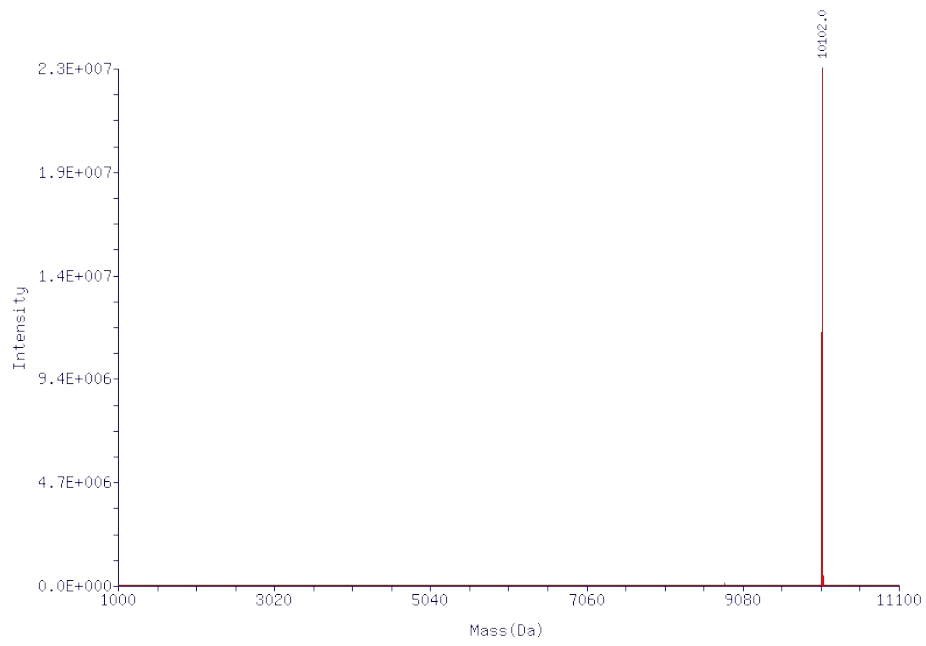
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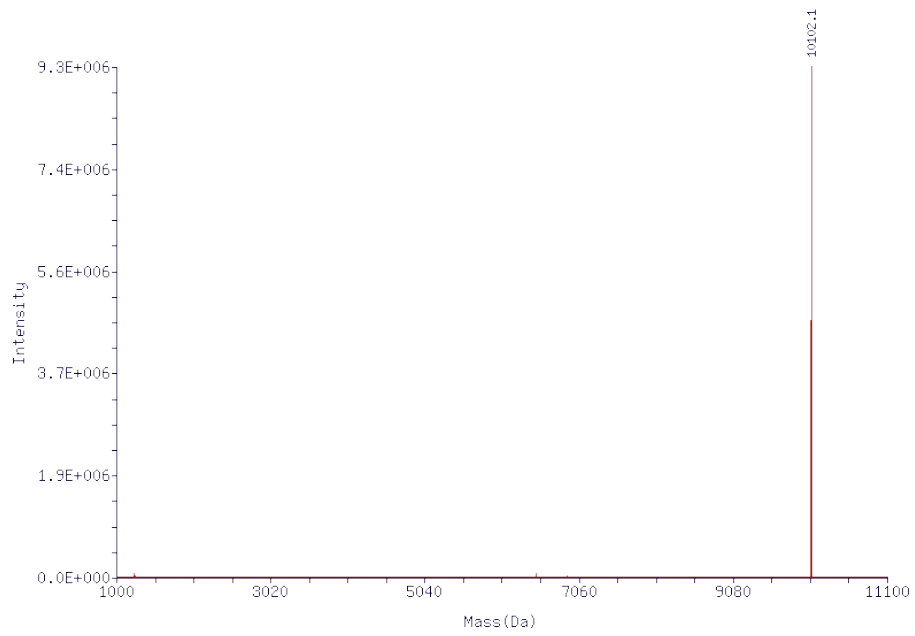
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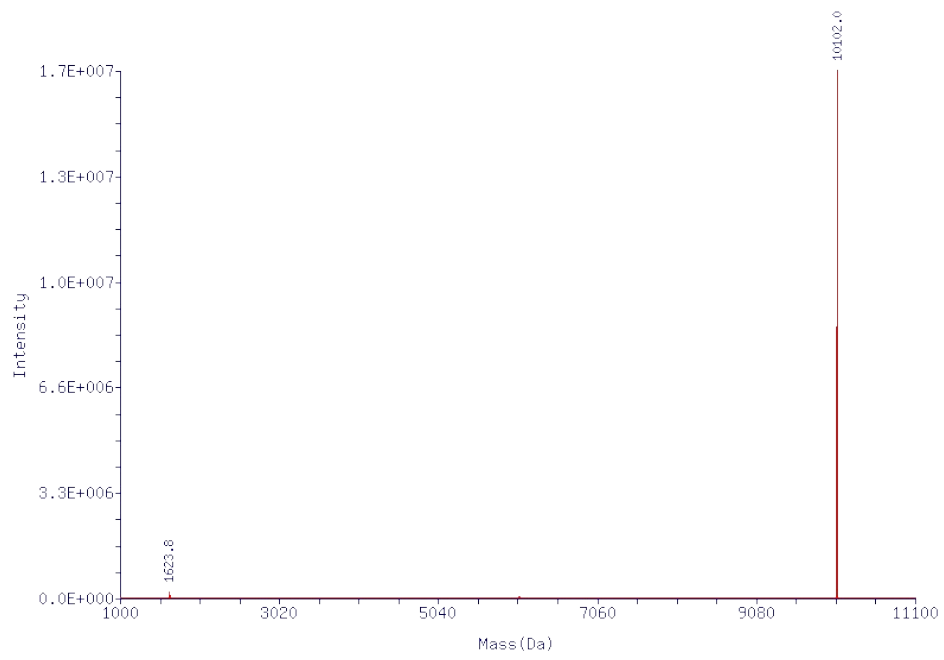
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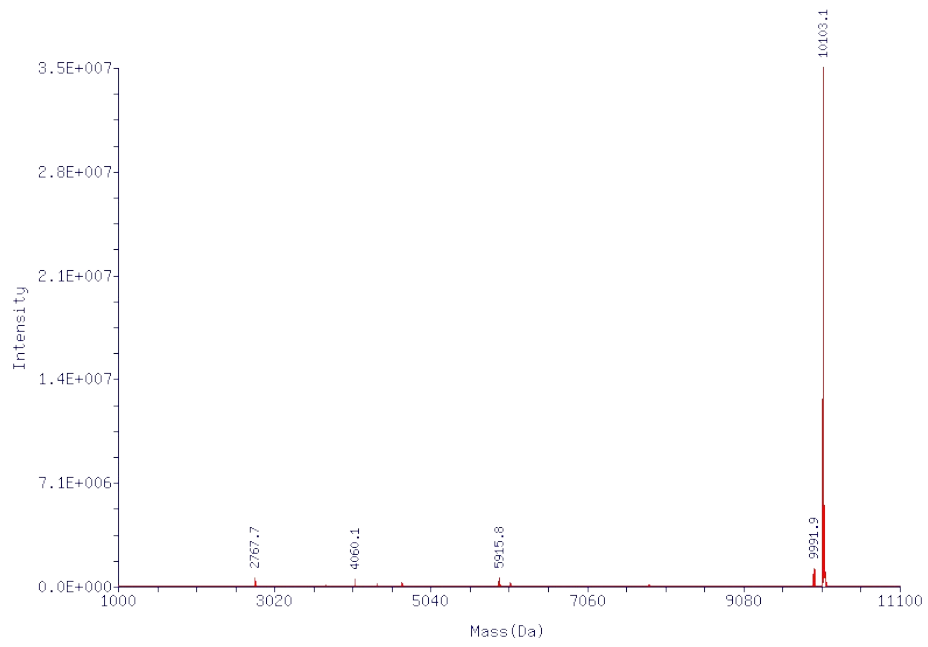


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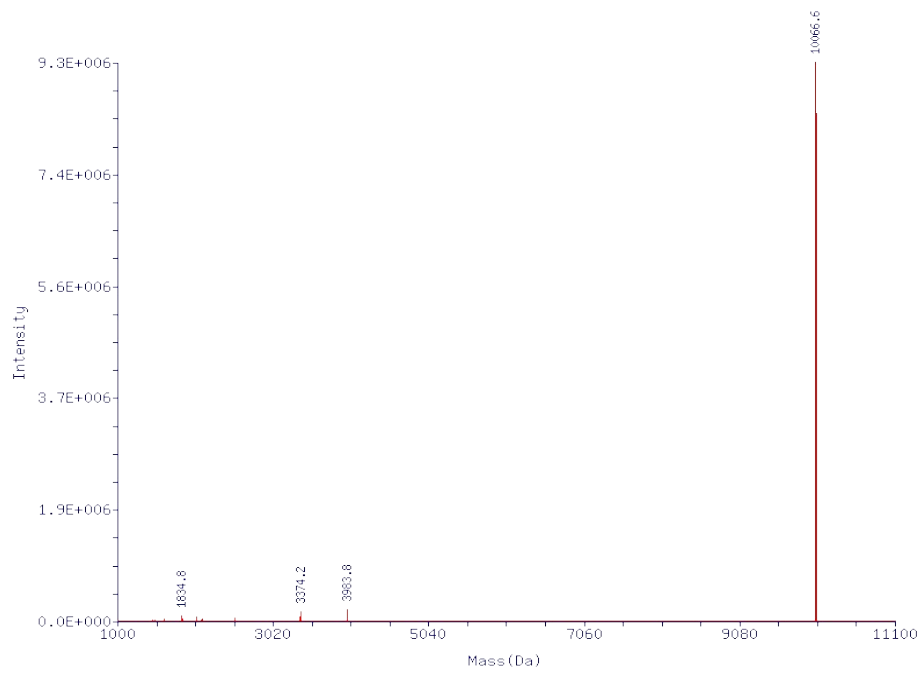
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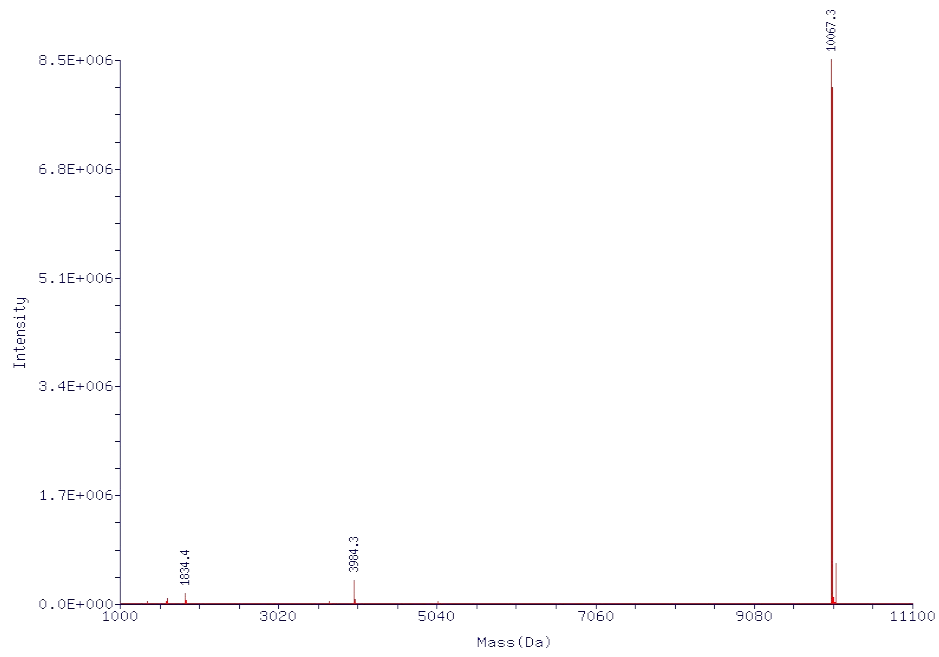


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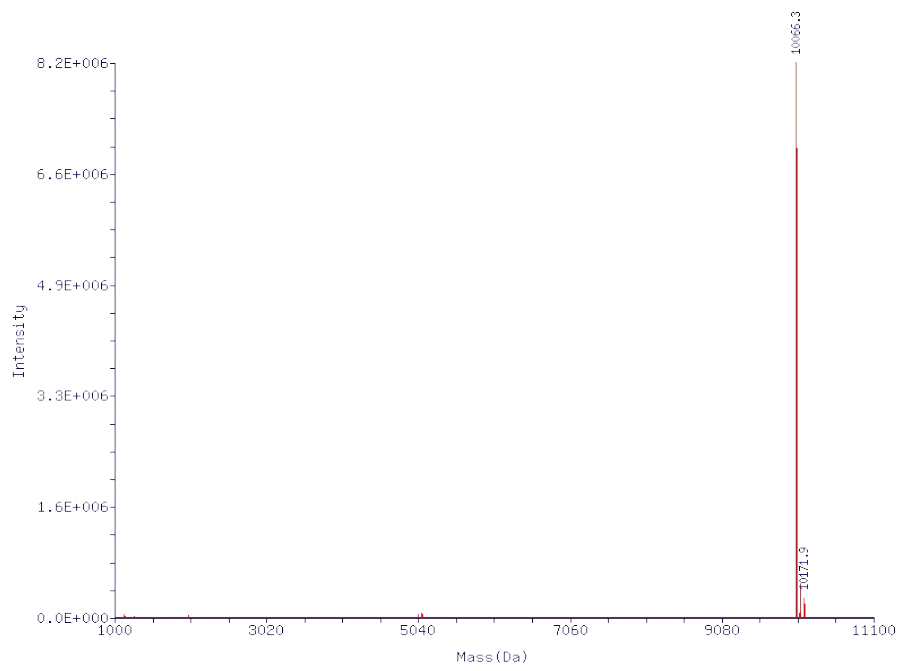




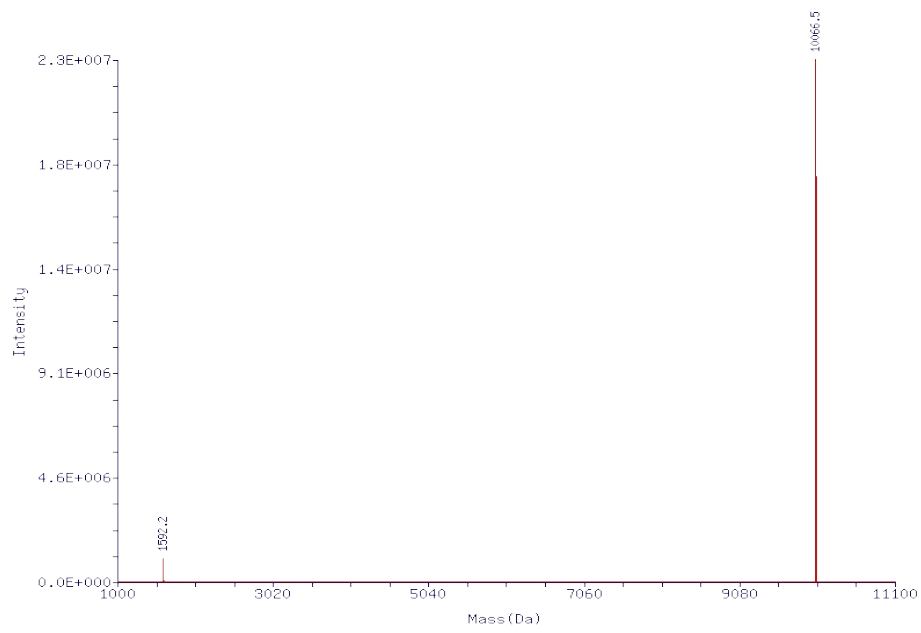
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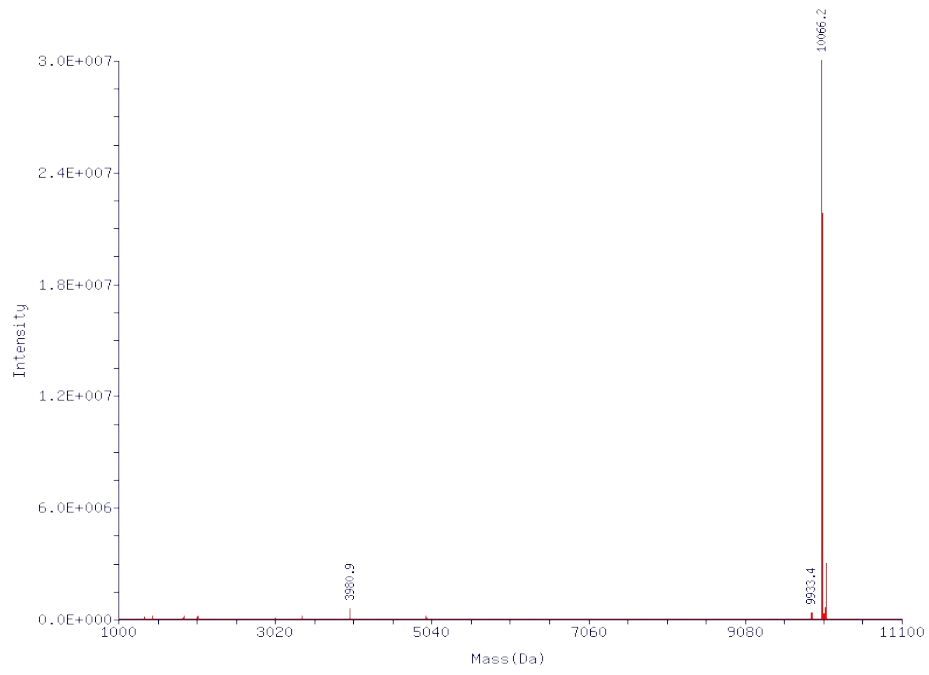
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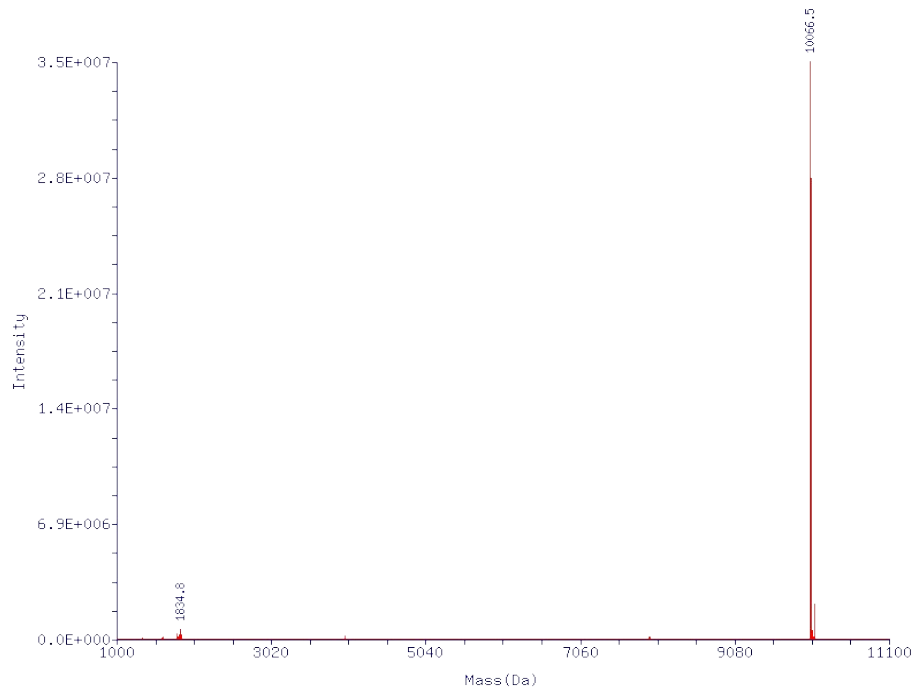
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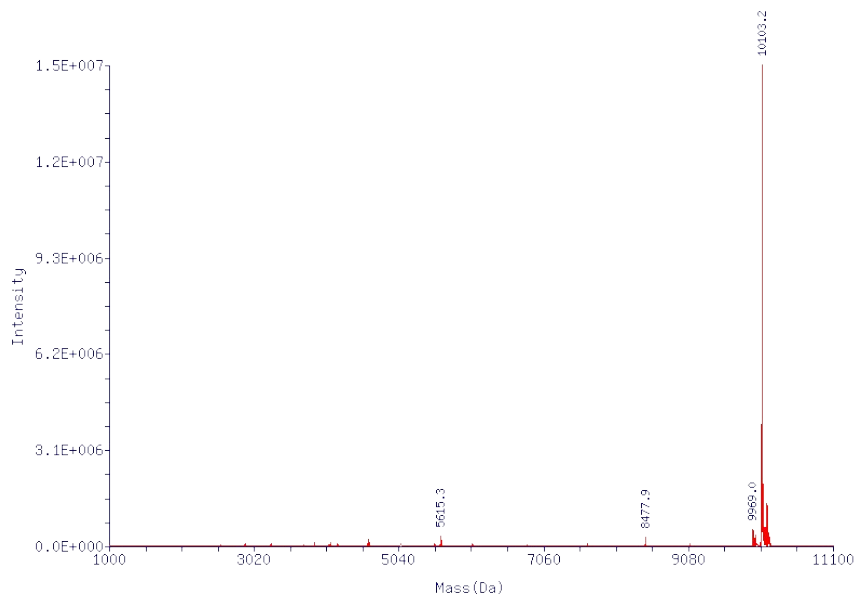
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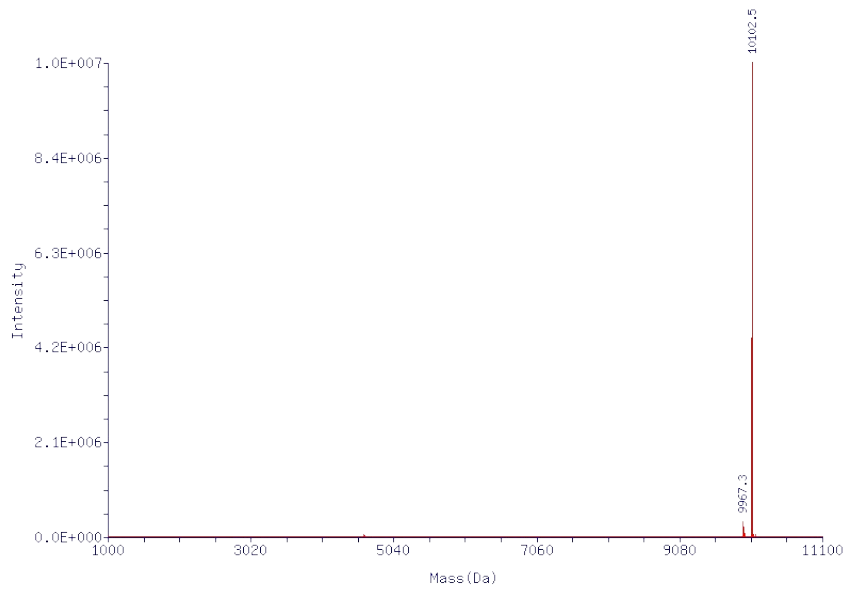
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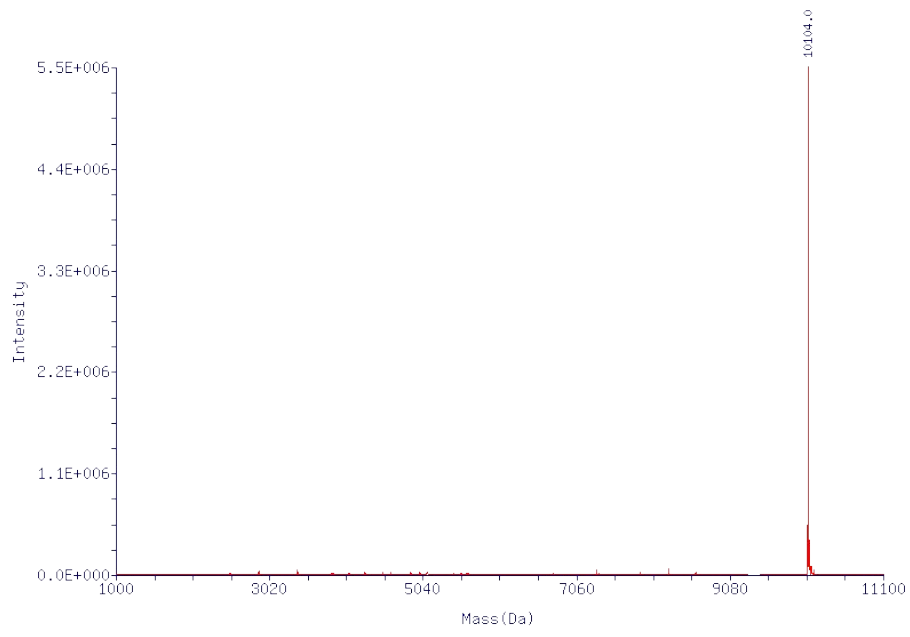
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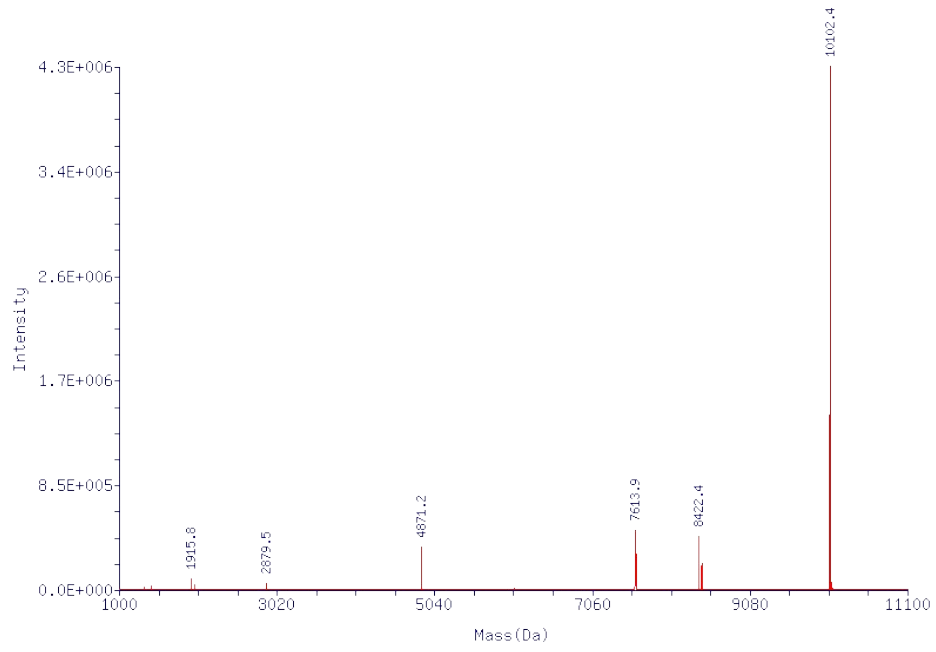
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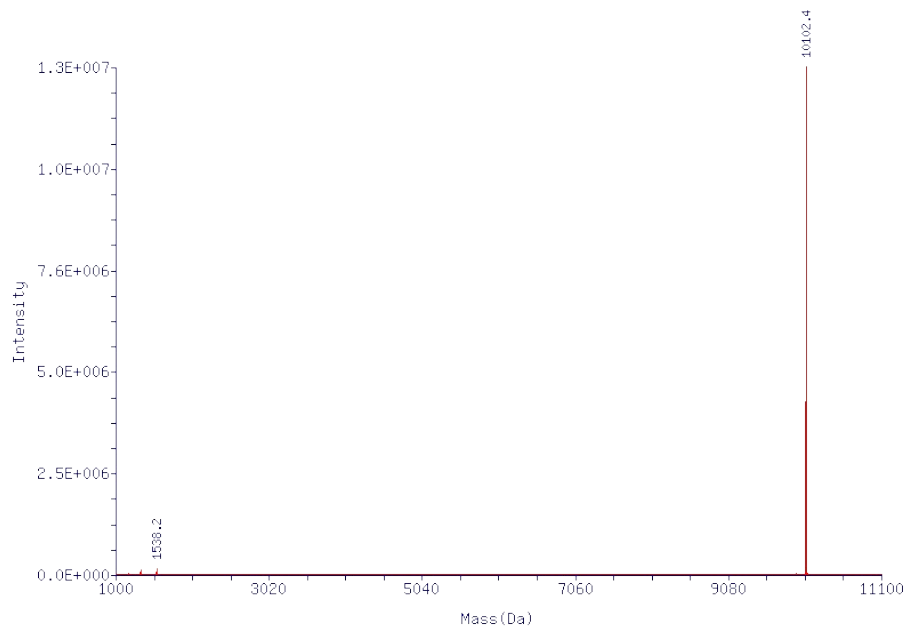
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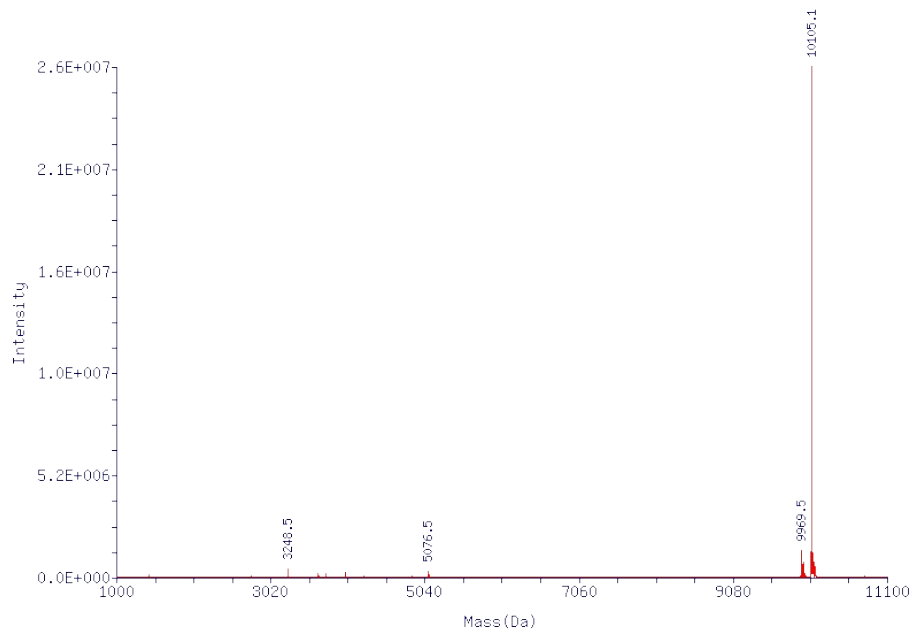
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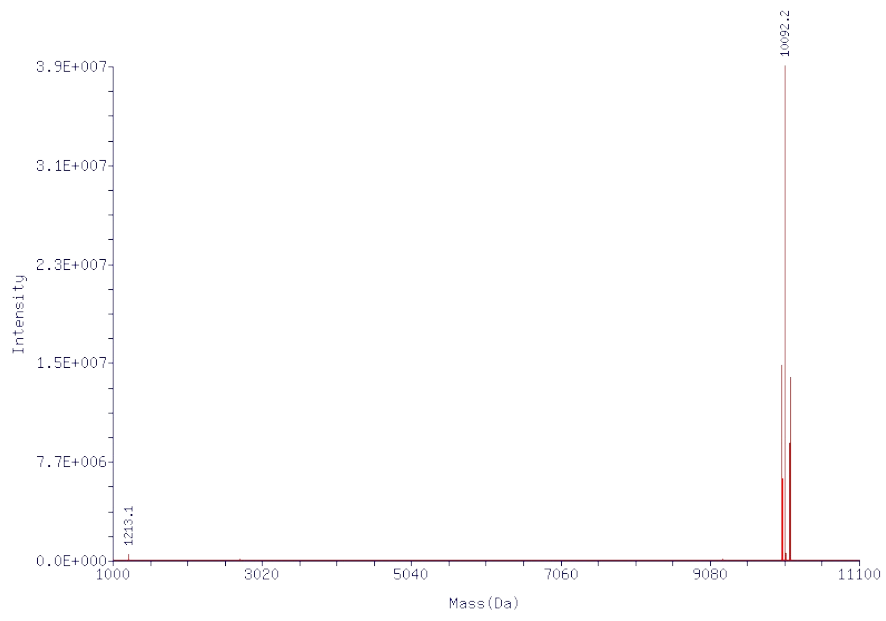
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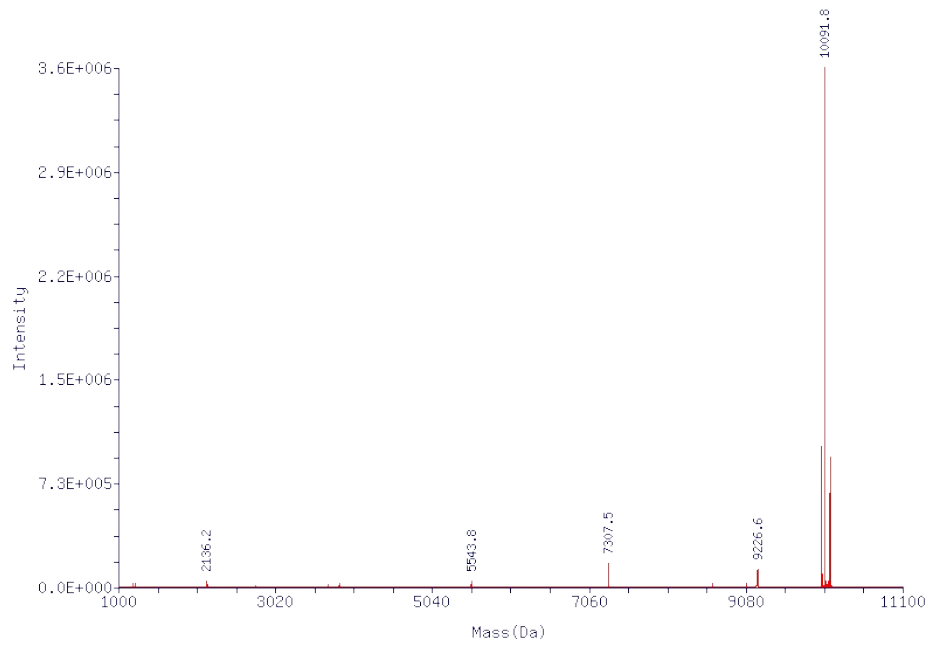
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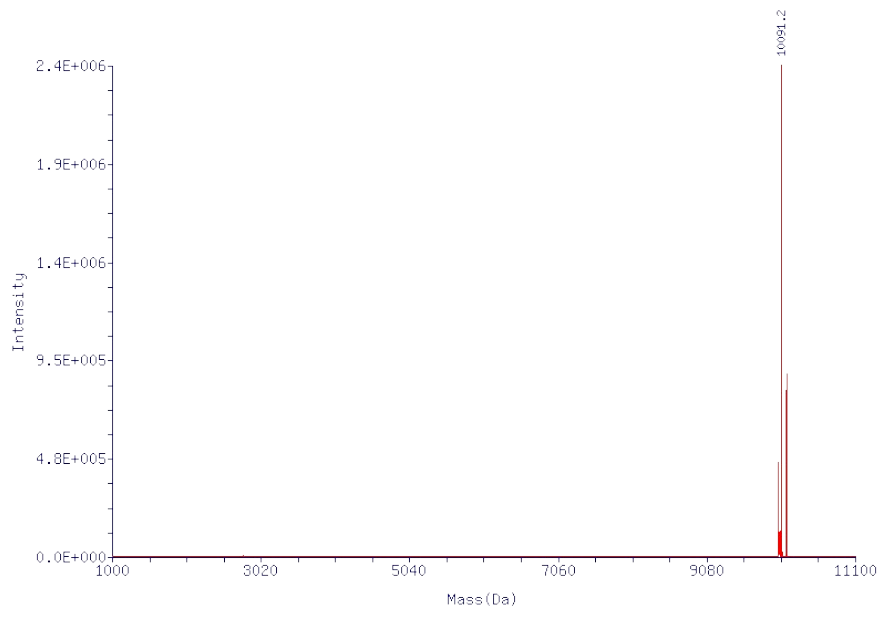
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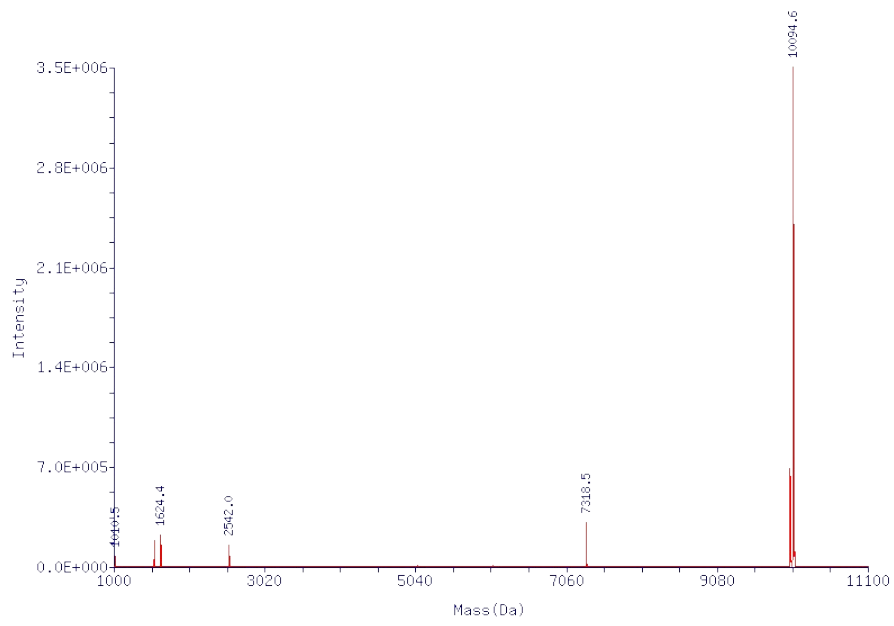
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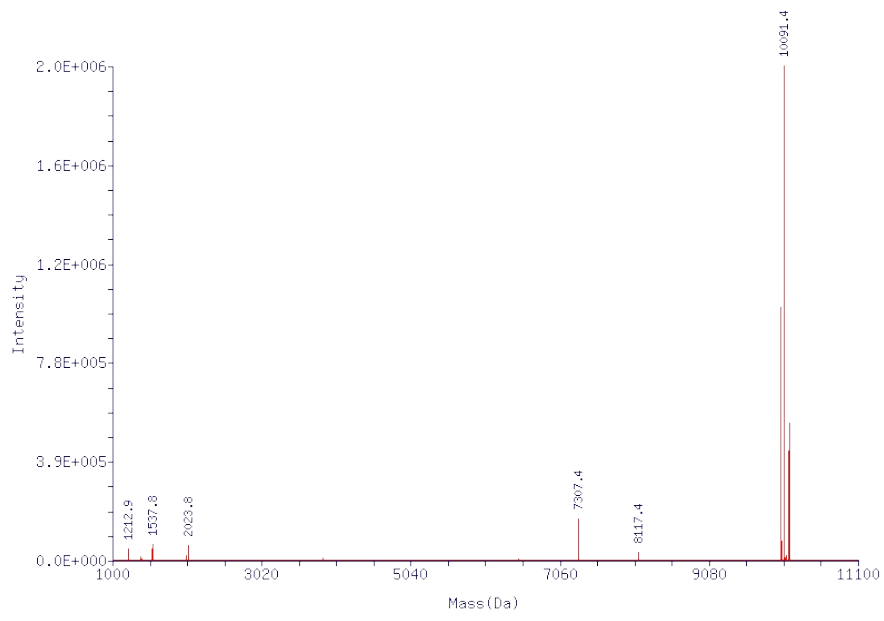


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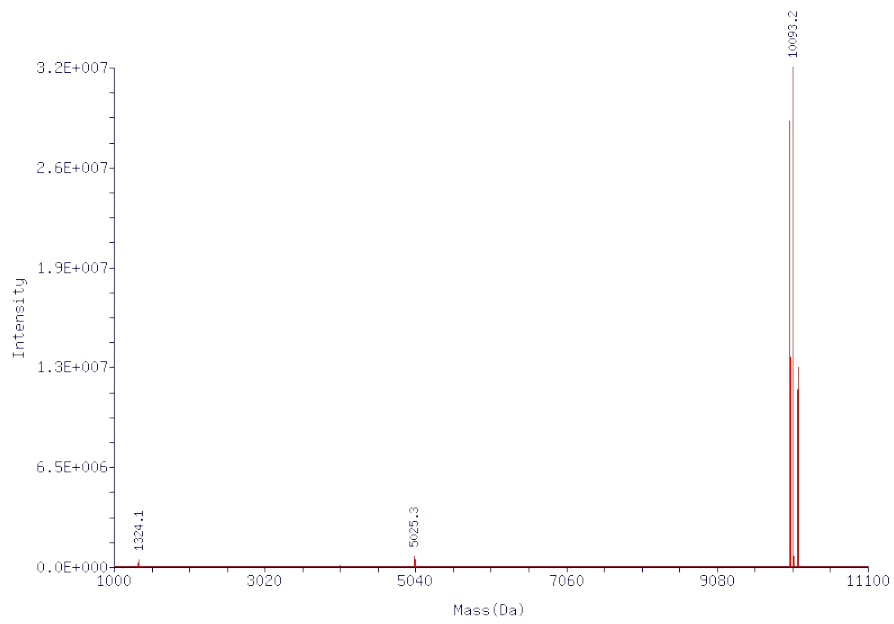


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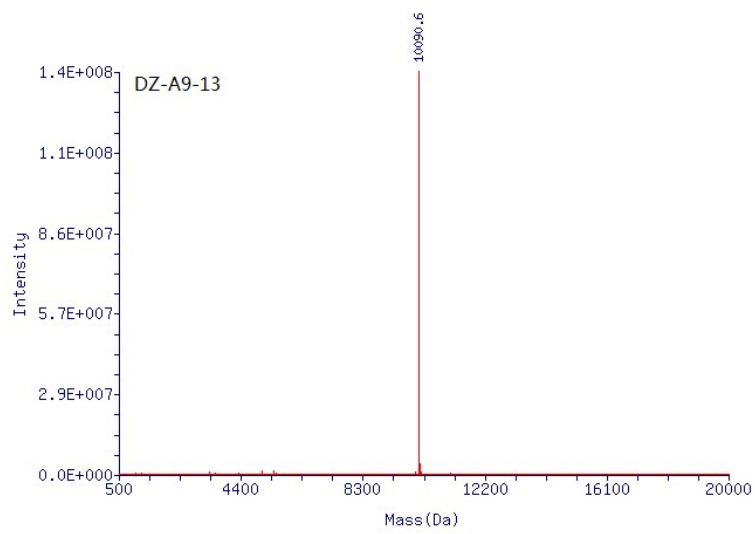


Fig. S2 MS spectra of DNAzymes with ESI-MS