

Supplement information:

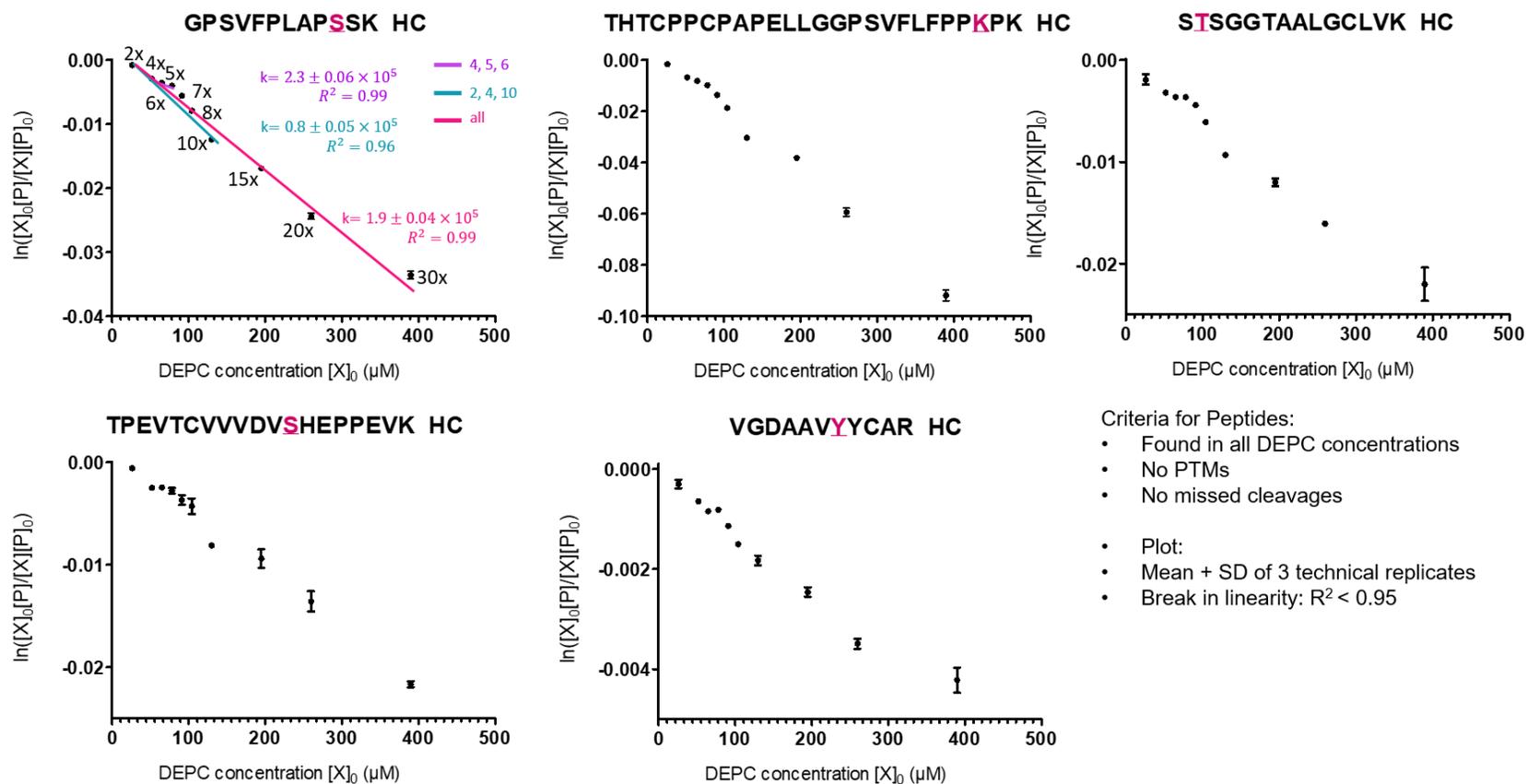


Figure S1: Reaction kinetics of DEPC labelling studied with dose response curve for different peptides of SILuMAb. The X axis is initial concentration of DEPC while Y axis is the ratio of DEPC modified peak area. Criteria for break in linearity was $R^2 < 0.95$ for the peptides assessed in our CL-MS study. HC signifies heavy chain.

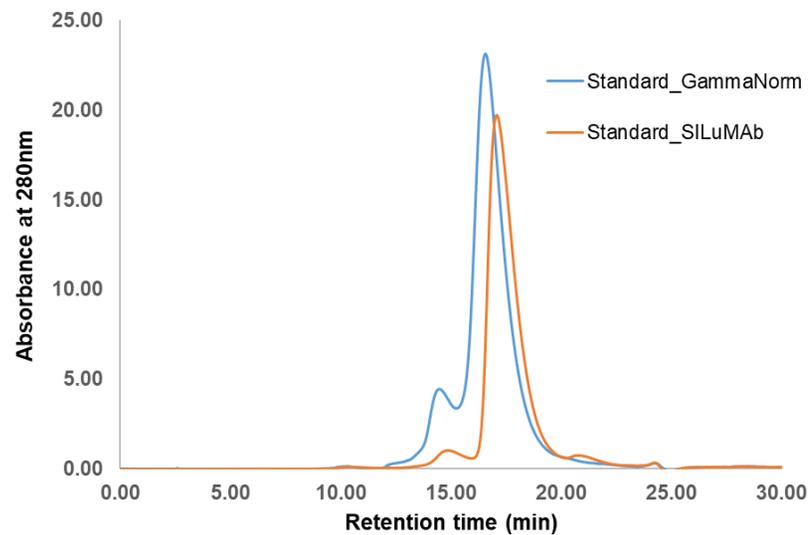


Figure S2: SEC-UV analysis of standard mAb – GammaNorm in blue and standard SILuMAb in orange. The monomer peak is seen to elute at 17-19 min, while the dimer peak is seen as small peak upfront of monomer at RT of 15 min. The standard SILuMAb also shows a minor fragment peak at 21 min. A small bump at 10 min is a column contamination visible in all samples and blanks.

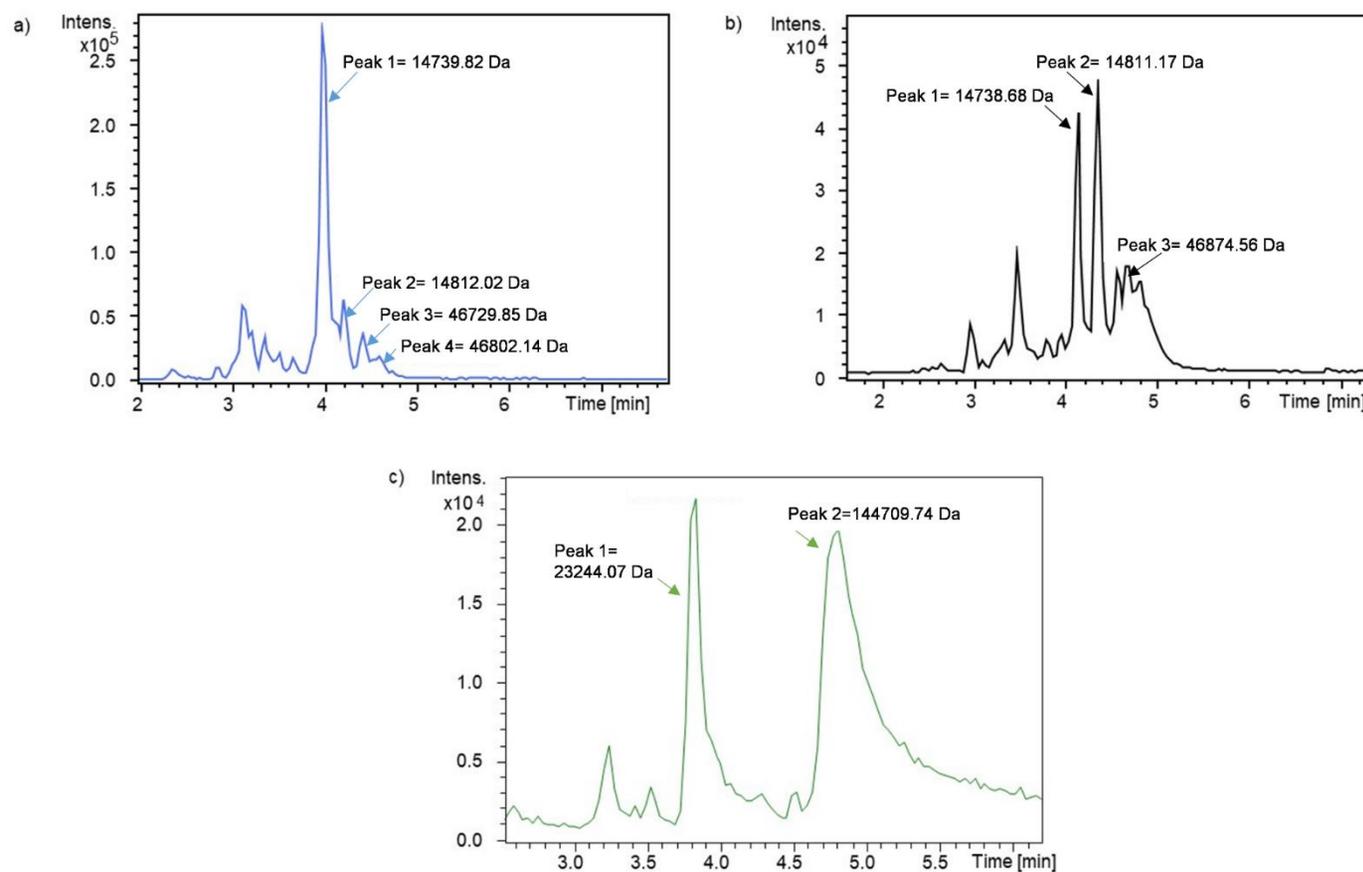


Figure S3: Chromatograms of RPLC-MS analysis for intact DEPC labeled long-term stored SILuMAb a) S₂yr sample with overhead annotation of the peak number and the mass (Da) detected at that specific RT. No monomeric peak is detected. b) S₁yr sample with overhead annotation of the peak number and the mass (Da) detected at that specific RT. Monomer mass is not detected. c) S₀yr sample with 2 peaks, and respective overhead annotations of the masses under these peaks. The second peak denotes the mass of deglycosylated and DEPC labeled monomer

Table S1: Logarithmised (log 2) intensity values for the sample set of heat stressed fresh SILuMAB (SH) and non-heat stressed SILuMAB (SO). NaN denotes missing value. The positions common with the heat stressed one-year stored SILuMAB vs non –heat stressed one year stored SILuMAB experiment (supporting table 3) are highlighted in bold & italics.

| Log ₂ intensity SH_1 | Log ₂ intensity SH_2 | Log ₂ intensity SH_3 | Log ₂ intensity SH_4 | Log ₂ intensity SO_1 | Log ₂ intensity SO_2 | Log ₂ intensity SO_3 | Log ₂ intensity SO_4 | Mod. Names | Mod . AAs | AA position | Protein chain |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------------|-----------------|-------------------|--------------------------------|
| 19.95 | NaN | 20.68 | 21.07 | 21.21 | 21.22 | 22.28 | 21.55 | DEPC/72.0 2 | H | 208 | SILu MAb- HC |
| NaN | 21.39 | 21.15 | NaN | 21.00 | NaN | 19.89 | NaN | DEPC/72.0 2 | H | 272 | SILu MAb- HC |
| <i>NaN</i> | <i>20.55</i> | <i>22.07</i> | <i>20.44</i> | <i>20.69</i> | <i>19.16</i> | <i>NaN</i> | <i>NaN</i> | <i>DEPC/72.0 2</i> | <i>H</i> | <i>289</i> | <i>SILu MAb- HC</i> |
| 19.64 | 22.28 | 22.44 | 22.66 | 21.74 | 19.97 | NaN | NaN | DEPC/72.0 2 | H | 314 | SILu MAb- HC |
| <i>21.98</i> | <i>22.90</i> | <i>23.23</i> | <i>23.13</i> | <i>22.52</i> | <i>20.40</i> | <i>21.79</i> | <i>NaN</i> | <i>DEPC/72.0 2</i> | <i>K</i> | <i>43</i> | <i>SILu MAb- HC</i> |
| <i>21.07</i> | <i>22.37</i> | <i>21.89</i> | <i>21.80</i> | <i>21.61</i> | <i>20.97</i> | <i>20.82</i> | <i>NaN</i> | <i>DEPC/72.0 2</i> | <i>K</i> | <i>44</i> | <i>SILu MAb- LC</i> |
| <i>19.37</i> | <i>20.89</i> | <i>20.13</i> | <i>18.66</i> | <i>19.15</i> | <i>18.83</i> | <i>NaN</i> | <i>NaN</i> | <i>DEPC/72.0 2</i> | <i>K</i> | <i>47</i> | <i>SILu MAb- LC</i> |
| <i>22.43</i> | <i>23.25</i> | <i>23.02</i> | <i>22.90</i> | <i>23.53</i> | <i>22.70</i> | <i>22.92</i> | <i>22.80</i> | <i>DEPC/72.0 2</i> | <i>K</i> | <i>50</i> | <i>SILu MAb- HC</i> |
| <i>22.12</i> | <i>22.00</i> | <i>21.85</i> | <i>22.47</i> | <i>22.02</i> | <i>22.10</i> | <i>21.01</i> | <i>21.00</i> | <i>DEPC/72.0 2</i> | <i>K</i> | <i>55</i> | <i>SILu MAb- LC</i> |

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|-------|-------|-------|-------|-------|-------|-------|-------|----------------|---|-----|--------------------|
| 22.68 | 22.91 | 23.19 | NaN | 23.72 | 23.75 | 22.95 | 22.97 | DEPC/72.0 2 | K | 64 | SILu MAb- HC |
| 19.97 | 23.59 | 21.58 | 21.47 | 21.79 | 20.29 | 20.73 | 19.82 | DEPC/72.0 2 | K | 75 | SILu MAb- HC |
| 20.70 | 21.55 | 20.70 | 21.02 | 21.22 | 19.97 | 20.69 | NaN | DEPC/72.0 2 | K | 116 | SILu MAb- LC |
| 19.91 | 21.97 | NaN | NaN | 21.00 | NaN | 19.84 | NaN | DEPC/72.0 2 | K | 135 | SILu MAb- LC |
| 20.65 | 20.82 | 21.53 | 20.33 | 22.37 | 21.87 | 21.20 | 21.00 | DEPC/72.0 2 | K | 162 | SILu MAb- LC |
| 19.27 | 19.82 | 21.08 | 21.10 | 20.69 | 20.92 | 19.88 | 18.92 | DEPC/72.0 2 | K | 172 | SILu MAb- LC |
| 20.62 | 21.52 | 21.83 | 21.29 | 21.11 | NaN | 20.67 | NaN | DEPC/72.0 2 | K | 177 | SILu MAb- LC |
| 21.87 | 22.39 | 22.02 | 22.08 | 22.32 | 22.14 | 22.60 | 20.68 | DEPC/72.0 2 | K | 192 | SILu MAb- LC |
| 19.19 | NaN | 21.04 | 20.25 | 21.80 | 20.48 | 21.31 | NaN | DEPC/72.0 2 | K | 209 | SILu MAb- HC |
| NaN | 22.16 | 22.50 | NaN | 23.04 | 19.39 | NaN | 18.45 | DEPC/72.0 2 | K | 226 | SILu MAb- HC |
| NaN | 20.32 | 20.81 | 21.09 | 21.34 | NaN | 19.63 | NaN | DEPC/72.0 2 | K | 252 | SILu MAb- HC |

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|-------|-------|-------|-------|-------|-------|-------|-------|----------------|---|-----|--------------------|
| 22.23 | 22.84 | 22.32 | 20.86 | 22.19 | 20.61 | 22.40 | 19.13 | DEPC/72.0 2 | K | 278 | SILu MAb- HC |
| 19.14 | 20.83 | 19.68 | 18.95 | 20.91 | 20.54 | 19.73 | 19.08 | DEPC/72.0 2 | K | 292 | SILu MAb- HC |
| 21.84 | 22.30 | 22.38 | 22.72 | 22.34 | 21.57 | 21.71 | 21.20 | DEPC/72.0 2 | K | 321 | SILu MAb- HC |
| 21.92 | 22.68 | 22.01 | 22.30 | 23.28 | 21.47 | 22.28 | NaN | DEPC/72.0 2 | K | 324 | SILu MAb- HC |
| NaN | 20.03 | 20.80 | NaN | 21.99 | 21.86 | 21.47 | NaN | DEPC/72.0 2 | K | 326 | SILu MAb- HC |
| 22.17 | 23.01 | 22.32 | NaN | 22.45 | 22.41 | 22.32 | 21.41 | DEPC/72.0 2 | K | 330 | SILu MAb- HC |
| 23.62 | 23.95 | 23.35 | 22.96 | 23.07 | 20.65 | 23.17 | NaN | DEPC/72.0 2 | K | 338 | SILu MAb- HC |
| 20.04 | 20.60 | 20.70 | NaN | 18.74 | 19.80 | NaN | 18.81 | DEPC/72.0 2 | K | 342 | SILu MAb- HC |
| 21.14 | 21.90 | 22.12 | NaN | 22.72 | 22.66 | NaN | 21.55 | DEPC/72.0 2 | K | 418 | SILu MAb- HC |
| 20.10 | NaN | 21.82 | NaN | 21.17 | 24.25 | NaN | NaN | DEPC/72.0 2 | S | 62 | SILu MAb- HC |
| 21.21 | 22.22 | 21.08 | 21.61 | 21.89 | NaN | 21.47 | NaN | DEPC/72.0 2 | S | 135 | SILu MAb- HC |

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|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|------------------------|----------|------------|-----------------------------|
| 21.21 | 22.22 | 21.93 | 21.61 | 21.89 | 20.80 | 21.47 | 19.30 | DEPC/72.0 2 | S | 136 | SILu MAb- HC |
| 18.62 | 19.28 | 18.71 | NaN | 19.16 | NaN | 19.38 | 19.22 | DEPC/72.0 2 | S | 159 | SILu MAb- LC |
| NaN | 20.75 | 22.05 | 21.19 | 22.73 | NaN | 20.62 | NaN | DEPC/72.0 2 | S | 171 | SILu MAb- LC |
| NaN | 22.65 | 21.25 | NaN | 22.63 | 20.76 | 20.86 | NaN | DEPC/72.0 2 | S | 188 | SILu MAb- HC |
| NaN | 21.05 | 19.64 | NaN | 19.84 | 19.50 | NaN | 20.12 | DEPC/72.0 2 | S | 198 | SILu MAb- LC |
| 19.26 | 20.04 | 19.88 | NaN | 19.81 | 19.62 | 18.95 | 18.56 | DEPC/72.0 2 | S | 258 | SILu MAb- HC |
| NaN | 23.18 | 20.80 | NaN | 21.82 | NaN | 22.18 | NaN | DEPC/72.0 2 | T | 191 | SILu MAb- HC |
| 20.59 | 21.97 | 21.87 | 20.81 | 19.33 | NaN | 18.88 | NaN | DEPC/72.0 2 | Y | 32 | SILu MAb- HC |
| NaN | NaN | 22.96 | 22.82 | 22.31 | 21.70 | NaN | NaN | DEPC/72.0 2 | Y | 32 | SILu MAb- LC |
| 21.27 | 20.91 | 21.25 | 21.65 | 21.17 | 21.44 | 21.01 | 21.02 | DEPC/72.0 2 | Y | 51 | SILu MAb- LC |
| NaN | NaN | 19.86 | 19.15 | NaN | 18.93 | 20.56 | NaN | DEPC/72.0 2 | Y | 183 | SILu MAb- LC |

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|-------|-------|-------|-------|-------|-------|-------|-------|-------------------------------|-----|-------|--------------------|
| 20.83 | 21.66 | 21.60 | 21.55 | 21.46 | 20.97 | 20.94 | 19.69 | DEPC/72.0 2 | Y | 282 | SILu MAb- HC |
| 20.79 | 23.38 | 23.06 | 22.88 | 23.08 | 21.37 | 22.36 | NaN | DEPC/72.0 2 | Y | 323 | SILu MAb- HC |
| NaN | 19.47 | 20.66 | 20.50 | 21.08 | NaN | 18.92 | 21.24 | DEPC/72.0 2;DEPC/72. 02 | K,S | 43,49 | SILu MAb- HC |
| 20.13 | 21.60 | 21.88 | 22.28 | 22.43 | 20.12 | 21.00 | NaN | DEPC/72.0 2;DEPC/72. 02 | K,S | 50,62 | SILu MAb- HC |
| NaN | NaN | 19.70 | 20.06 | 20.88 | 19.90 | NaN | NaN | DEPC/72.0 2;DEPC/72. 02 | S,K | 49,64 | SILu MAb- HC |

Table S2: Logarithmised (log 2) intensity values for the sample set of heat stressed one year stored SILuMAb (LS_H) and non –heat stressed one year stored SILuMAb (LS_O). NaN denotes missing value. The positions common with the heat stressed fresh SILuMAb vs non –heat stressed fresh SILuMAb experiment (supporting table 2) are highlighted in bold & italics.

| Log ₂ intensity LS_H_1 | Log ₂ intensity LS_H_2 | Log ₂ intensity LS_H_3 | Log ₂ intensity LS_H_4 | Log ₂ intensity LS_H_5 | Log ₂ intensity LS_H_6 | Log ₂ intensity LS_O_1 | Log ₂ intensity LS_O_2 | Log ₂ intensity LS_O_3 | Log ₂ intensity LS_O_4 | Log ₂ intensity LS_O_5 | Log ₂ intensity LS_O_6 | Mod. Names | Mod . AAs | AA position | Protein chain |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------|-----------|-------------|-------------------|
| 23.02 | 24.39 | 24.07 | 22.63 | 24.84 | 23.35 | 23.77 | 23.77 | 23.81 | 23.79 | 23.52 | 23.51 | DEPC/72.02 | H | 35 | SILuMAb-HC |
| 22.33 | 22.40 | 22.10 | 21.98 | 22.76 | 22.78 | 22.68 | 22.69 | 22.93 | 22.94 | 22.66 | 22.63 | DEPC/72.02 | H | 272 | SILuMAb-HC |
| 21.34 | 21.95 | 21.08 | 21.52 | 23.00 | 23.02 | 22.80 | 22.79 | 23.15 | 23.15 | 22.99 | 23.48 | DEPC/72.02 | H | 289 | SILuMAb-HC |
| 22.96 | 22.95 | 22.79 | 22.72 | 23.09 | 23.36 | 23.54 | 23.52 | 23.60 | 23.54 | 23.35 | 23.70 | DEPC/72.02 | H | 314 | SILuMAb-HC |
| 22.59 | 22.23 | 22.84 | 20.88 | 22.90 | 23.15 | 23.94 | 23.48 | 24.23 | 23.86 | 24.30 | 23.35 | DEPC/72.02 | H | 433 | SILuMAb-HC |
| 22.65 | 22.65 | NaN | NaN | 21.10 | NaN | 22.69 | 23.25 | 23.56 | 23.13 | 24.28 | 22.69 | DEPC/72.02 | H | 437 | SILuMAb-HC |
| 22.43 | NaN | NaN | 22.34 | 24.04 | 21.60 | NaN | 21.73 | 21.91 | 23.19 | NaN | 23.95 | DEPC/72.02 | H | 439 | SILuMAb-HC |
| 23.24 | 23.28 | 23.31 | 23.34 | 23.80 | 23.75 | 20.55 | 20.49 | 20.84 | 20.84 | 20.65 | 20.68 | DEPC/72.02 | K | 43 | SILuMAb-HC |
| 22.85 | 22.87 | 22.75 | 22.72 | 23.17 | 23.14 | 23.34 | 23.32 | 23.56 | 23.58 | 23.80 | 23.41 | DEPC/72.02 | K | 50 | SILuMAb-HC |
| 24.95 | 24.94 | 24.96 | 24.96 | 25.37 | 25.34 | 25.21 | 25.21 | 25.41 | 25.40 | 26.38 | 26.36 | DEPC/72.02 | K | 64 | SILuMAb-HC |
| 24.94 | 24.93 | 24.71 | 24.70 | 25.21 | 25.19 | 24.98 | 24.97 | 25.14 | 25.13 | 24.98 | 24.98 | DEPC/72.02 | K | 75 | SILuMAb-HC |

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|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------|-------------------|----------|------------|------------------------|
| 21.8 3 | 21.8 3 | 22.0 2 | 21.9 6 | 22.5 6 | 22.2 2 | 22. 96 | 22. 98 | 22. 68 | 22. 63 | 22.4 8 | 22.45 | DEPC/72.02 | K | 125 | SILuMAb- HC |
| 25.0 6 | 25.0 5 | 25.0 6 | 24.5 3 | 25.3 9 | 25.1 3 | 25. 16 | 25. 17 | 25. 07 | 25. 46 | 24.4 4 | 25.38 | DEPC/72.02 | K | 137 | SILuMAb- HC |
| 20.6 8 | 20.3 2 | 20.9 0 | 20.5 9 | 21.0 5 | 21.0 0 | 21. 65 | 21. 10 | 21. 51 | 21. 37 | 21.3 9 | 21.39 | DEPC/72.02 | K | 250 | SILuMAb- HC |
| 21.4 3 | 20.9 7 | 20.4 7 | 21.0 5 | 22.1 5 | 21.7 7 | 22. 05 | 22. 04 | 22. 11 | 22. 10 | 22.1 7 | 22.15 | DEPC/72.02 | K | 252 | SILuMAb- HC |
| 21.1 0 | 20.9 2 | 20.6 9 | 20.8 3 | 21.0 2 | 21.0 2 | 21. 58 | 21. 52 | 21. 40 | 21. 49 | 21.3 1 | 21.44 | DEPC/72.02 | K | 278 | SILuMAb- HC |
| 20.3 4 | 20.2 7 | 20.7 2 | 20.2 5 | 21.1 5 | 21.4 4 | 21. 70 | 21. 27 | 21. 58 | 22. 04 | 21.5 8 | 21.88 | DEPC/72.02 | K | 292 | SILuMAb- HC |
| 23.1 0 | 23.1 1 | 23.4 4 | 23.3 0 | 23.6 4 | 23.4 0 | 23. 35 | 23. 85 | 23. 21 | 23. 21 | 22.9 6 | 22.95 | DEPC/72.02 | K | 321 | SILuMAb- HC |
| 22.1 6 | 22.2 2 | 22.1 2 | 22.1 6 | 22.8 2 | 22.8 5 | 23. 06 | 23. 07 | 22. 99 | 22. 99 | 22.8 6 | 22.86 | DEPC/72.02 | K | 324 | SILuMAb- HC |
| 22.1 8 | 20.9 0 | <i>NaN</i> | <i>NaN</i> | 22.5 6 | 22.3 8 | <i>NaN</i> | <i>NaN</i> | 23. 39 | 22. 77 | <i>NaN</i> | 22.70 | DEPC/72.02 | K | 326 | SILuMAb- HC |
| 23.3 5 | 24.2 8 | 23.5 6 | 23.5 5 | 23.4 6 | 23.4 3 | 24. 35 | 23. 88 | 24. 52 | 24. 02 | 24.1 2 | 24.13 | DEPC/72.02 | K | 330 | SILuMAb- HC |
| 24.6 5 | 24.6 6 | 24.9 7 | 24.9 8 | 25.2 6 | 25.2 4 | 25. 67 | 25. 67 | 25. 82 | 25. 82 | 25.8 1 | 25.81 | DEPC/72.02 | K | 338 | SILuMAb- HC |
| <i>NaN</i> | 19.9 5 | 20.6 4 | 20.4 4 | 21.3 1 | 21.2 1 | 21. 59 | 21. 72 | 21. 96 | 22. 36 | 22.6 3 | 22.60 | DEPC/72.02 | K | 342 | SILuMAb- HC |
| 24.7 1 | 24.4 2 | 24.8 0 | 24.4 0 | 24.9 1 | 24.8 9 | 24. 56 | 24. 47 | 24. 19 | 24. 68 | 24.6 5 | 24.38 | DEPC/72.02 | K | 364 | SILuMAb- HC |
| 22.5 6 | 22.5 5 | 22.7 5 | 22.6 3 | 23.3 9 | 23.2 7 | 24. 42 | 23. 68 | 23. 43 | 23. 34 | 23.1 6 | 23.10 | DEPC/72.02 | K | 396 | SILuMAb- HC |
| 28.2 4 | 28.2 4 | 28.3 0 | 28.3 0 | 28.6 3 | 28.6 1 | 28. 41 | 28. 41 | 28. 55 | 28. 55 | 28.5 7 | 28.56 | DEPC/72.02 | K | 418 | SILuMAb- HC |
| 21.7 3 | <i>NaN</i> | 23.1 7 | 23.1 6 | <i>NaN</i> | 23.1 6 | <i>NaN</i> | 23. 04 | 23. 94 | <i>NaN</i> | 23.3 1 | 23.18 | DEPC/72.02 | K | 443 | SILuMAb- HC |

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|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------|-------------------|----------|------------|------------------------|
| 21.0 2 | 20.9 6 | 21.1 0 | 21.0 1 | 21.4 1 | 21.1 8 | 21. 59 | 21. 63 | NaN | 21. 72 | NaN | 21.79 | DEPC/72.02 | S | 128 | SILuMAb- HC |
| NaN | 20.9 6 | NaN | 25.7 6 | NaN | 21.1 8 | NaN | 25. 78 | NaN | NaN | 21.7 9 | 21.79 | DEPC/72.02 | S | 135 | SILuMAb- HC |
| 21.0 2 | NaN | 21.1 0 | NaN | 23.3 1 | NaN | 21. 59 | NaN | NaN | NaN | 22.4 6 | 23.63 | DEPC/72.02 | S | 136 | SILuMAb- HC |
| NaN | 20.9 7 | NaN | 21.0 5 | 21.7 9 | NaN | 21. 64 | 21. 61 | 20. 00 | 19. 97 | 22.4 4 | 21.67 | DEPC/72.02 | S | 258 | SILuMAb- HC |
| 22.3 3 | NaN | 21.7 6 | NaN | NaN | 22.7 8 | NaN | NaN | 22. 93 | 22. 94 | 22.6 6 | 22.63 | DEPC/72.02 | S | 271 | SILuMAb- HC |
| 22.3 5 | 20.3 3 | 23.3 9 | NaN | 21.1 4 | 20.8 7 | 21. 30 | 21. 32 | 23. 02 | NaN | 22.2 6 | 21.95 | DEPC/72.02 | T | 441 | SILuMAb- HC |
| 23.4 6 | 22.7 9 | 23.3 9 | 22.5 4 | 23.7 7 | 23.2 4 | 22. 89 | 22. 40 | 22. 44 | 22. 91 | 23.5 2 | 23.51 | DEPC/72.02 | Y | 32 | SILuMAb- HC |
| NaN | 20.3 2 | 19.9 6 | NaN | 20.4 9 | 20.4 9 | NaN | 20. 02 | 20. 16 | 20. 31 | 19.9 7 | NaN | DEPC/72.02 | Y | 79 | SILuMAb- HC |
| 22.6 1 | 22.6 4 | 22.6 3 | 22.6 7 | 23.2 0 | 23.1 8 | 21. 80 | 21. 79 | 21. 98 | 21. 97 | 21.9 2 | 21.95 | DEPC/72.02 | Y | 93 | SILuMAb- HC |
| 21.1 8 | 21.2 9 | 21.2 5 | 21.2 1 | 21.7 5 | 21.8 1 | 21. 74 | 22. 16 | 22. 52 | 22. 53 | 22.4 0 | 22.39 | DEPC/72.02 | Y | 282 | SILuMAb- HC |
| 21.6 3 | 21.6 5 | 21.8 6 | 21.5 7 | 22.1 4 | 22.1 8 | 22. 56 | 22. 63 | 24. 22 | 24. 21 | 23.9 8 | 23.09 | DEPC/72.02 | Y | 323 | SILuMAb- HC |
| 22.0 8 | NaN | 21.7 6 | NaN | NaN | 21.6 8 | 22. 24 | 22. 24 | NaN | 22. 26 | 22.2 0 | 22.23 | DEPC/72.02 | Y | 377 | SILuMAb- HC |
| 22.0 5 | NaN | 22.6 2 | 23.3 9 | 24.3 4 | 23.7 3 | 23. 44 | 23. 30 | 22. 90 | 22. 63 | 22.7 7 | 23.45 | DEPC/72.02 | Y | 440 | SILuMAb- HC |
| 24.0 3 | 21.7 0 | 23.7 0 | 20.8 9 | 24.0 7 | 24.1 9 | 24. 53 | 20. 61 | 24. 48 | 24. 48 | 20.4 1 | 24.43 | DEPC/72.02 | H | 41 | SILuMAb- LC |
| NaN | 23.7 1 | 23.4 6 | 23.4 6 | 23.8 0 | 23.8 5 | 23. 78 | 23. 76 | NaN | 24. 43 | NaN | 24.68 | DEPC/72.02 | H | 203 | SILuMAb- LC |
| 23.6 6 | 23.6 6 | 23.4 3 | 23.4 2 | 24.4 5 | 23.9 2 | 24. 19 | 24. 20 | 24. 22 | 24. 20 | 24.1 5 | 24.15 | DEPC/72.02 | K | 44 | SILuMAb- LC |

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|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------------------------|-----|-------------|------------|
| 22.69 | 22.71 | 22.78 | 22.76 | 23.18 | 22.85 | 22.53 | 22.47 | 22.69 | 22.64 | 23.07 | 23.06 | DEPC/72.02 | K | 47 | SILuMAb-LC |
| 23.20 | 23.21 | 23.73 | 23.02 | 23.27 | 23.27 | 22.22 | 23.80 | 22.81 | 23.89 | 23.84 | 24.03 | DEPC/72.02 | K | 55 | SILuMAb-LC |
| 21.38 | 21.88 | 21.89 | NaN | NaN | NaN | 22.35 | 22.74 | 22.15 | 22.48 | NaN | 22.20 | DEPC/72.02 | K | 68 | SILuMAb-LC |
| 24.17 | 24.56 | 23.73 | 23.72 | 24.23 | 24.54 | 24.07 | 24.07 | 24.38 | 24.36 | 24.19 | 24.17 | DEPC/72.02 | K | 116 | SILuMAb-LC |
| NaN | NaN | 21.61 | 21.51 | 22.27 | 21.48 | 22.66 | 22.64 | NaN | 21.98 | 21.74 | 21.60 | DEPC/72.02 | K | 135 | SILuMAb-LC |
| NaN | NaN | 20.31 | 20.34 | 20.65 | NaN | 20.70 | 20.79 | 20.61 | 20.61 | 20.54 | 20.43 | DEPC/72.02 | K | 155 | SILuMAb-LC |
| 23.68 | 23.69 | 23.59 | 23.62 | 24.08 | 24.08 | 23.87 | 23.89 | 23.88 | 23.87 | 24.35 | 24.35 | DEPC/72.02 | K | 162 | SILuMAb-LC |
| 22.27 | 22.26 | 22.11 | 22.21 | 22.63 | 21.63 | 23.11 | 23.10 | 23.25 | 23.25 | 23.16 | 23.24 | DEPC/72.02 | K | 177 | SILuMAb-LC |
| 23.73 | 23.70 | 23.93 | 23.54 | 24.04 | 24.07 | 24.48 | 24.14 | 24.04 | 24.36 | 24.42 | 24.42 | DEPC/72.02 | K | 192 | SILuMAb-LC |
| 23.74 | 23.75 | 23.38 | 23.36 | 23.82 | 23.78 | 23.80 | 23.82 | 23.99 | 23.98 | 24.15 | 24.15 | DEPC/72.02 | K | 210 | SILuMAb-LC |
| NaN | NaN | 20.00 | 20.04 | 20.36 | 20.44 | 19.97 | 20.04 | 20.70 | 21.41 | 22.23 | 21.74 | DEPC/72.02 | K | 218 | SILuMAb-HC |
| 21.22 | 21.23 | 23.60 | 20.89 | 23.94 | 20.27 | 24.40 | 24.44 | 24.32 | 24.38 | 24.25 | 24.42 | DEPC/72.02 | Y | 32 | SILuMAb-LC |
| 24.86 | 20.53 | 24.56 | 24.57 | 20.84 | 25.21 | 25.30 | 21.49 | 25.43 | 21.44 | 21.42 | 25.34 | DEPC/72.02 | Y | 38 | SILuMAb-LC |
| 23.15 | 23.14 | 23.50 | 23.49 | 23.66 | 23.66 | 23.73 | 24.11 | 24.30 | 24.30 | 24.00 | 24.43 | DEPC/72.02 | Y | 51 | SILuMAb-LC |
| 22.65 | 22.59 | 22.64 | 22.64 | 22.09 | 22.39 | 22.04 | 22.01 | 21.83 | 22.17 | 22.92 | 22.85 | DEPC/72.02; DEPC/72.02 | H,K | 314, 321 | SILuMAb-HC |
| 22.46 | NaN | 22.34 | NaN | 23.18 | 21.15 | 21.67 | 22.83 | 22.75 | NaN | NaN | 21.27 | DEPC/72.02; DEPC/72.02 | H,K | 314, 324 | SILuMAb-HC |

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|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------|---------------------------|-----|-------------|----------------|
| 22.8 1 | 21.8 8 | 22.9 8 | 22.3 6 | 22.0 0 | 24.3 1 | 22. 46 | 23. 84 | 23. 66 | 23. 34 | 24.1 0 | 23.56 | DEPC/72.02; DEPC/72.02 | H,Y | 433, 440 | SILuMAb- HC |
| 21.7 6 | 21.3 4 | 20.1 0 | NaN | 20.4 4 | 24.1 0 | 24. 30 | 20. 51 | NaN | 22. 48 | 23.1 3 | NaN | DEPC/72.02; DEPC/72.02 | H,Y | 437, 440 | SILuMAb- HC |
| 22.0 2 | 22.4 9 | NaN | NaN | 22.4 2 | 24.2 8 | 22. 21 | NaN | 22. 01 | 24. 47 | 24.4 6 | NaN | DEPC/72.02; DEPC/72.02 | H,Y | 439, 440 | SILuMAb- HC |
| 22.1 9 | 22.2 0 | 22.6 2 | 21.7 7 | 22.6 6 | 22.6 4 | 22. 74 | 22. 77 | 22. 77 | 22. 76 | 22.3 9 | 22.42 | DEPC/72.02; DEPC/72.02 | K,S | 43,4 9 | SILuMAb- HC |
| 24.5 5 | 24.5 2 | 24.9 8 | 24.2 0 | 22.9 2 | NaN | 25. 20 | 24. 75 | 25. 26 | 25. 24 | 24.5 6 | 25.69 | DEPC/72.02; DEPC/72.02 | K,S | 50,6 2 | SILuMAb- HC |
| 20.8 1 | 22.5 4 | 21.3 0 | 21.2 9 | 21.7 8 | 21.7 8 | 22. 94 | 21. 79 | 22. 09 | NaN | 22.8 4 | 22.13 | DEPC/72.02; DEPC/72.02 | K,S | 324, 328 | SILuMAb- HC |
| 25.5 2 | 25.5 2 | 24.5 4 | 25.2 6 | 25.4 8 | 24.7 6 | 25. 41 | 25. 42 | 25. 50 | NaN | 25.4 8 | 25.94 | DEPC/72.02; DEPC/72.02 | K,S | 338, 341 | SILuMAb- HC |
| 21.7 1 | 21.6 7 | 21.8 2 | 21.8 3 | 22.1 8 | 22.1 1 | 21. 90 | 21. 89 | 21. 91 | 21. 92 | 21.9 7 | 21.96 | DEPC/72.02; DEPC/72.02 | K,S | 418, 419 | SILuMAb- HC |
| 21.5 3 | 21.3 8 | 21.1 3 | 20.9 3 | 22.1 5 | 22.0 1 | 21. 77 | 21. 98 | 21. 85 | 21. 61 | 21.3 3 | 21.49 | DEPC/72.02; DEPC/72.02 | K,T | 250, 254 | SILuMAb- HC |
| 22.6 7 | 22.6 9 | 22.9 9 | 23.0 1 | 22.5 9 | 23.0 3 | 23. 13 | 23. 40 | 23. 58 | 23. 56 | 23.5 4 | 22.76 | DEPC/72.02; DEPC/72.02 | K,T | 330, 339 | SILuMAb- HC |
| 22.1 4 | 22.1 6 | 21.8 2 | 21.8 3 | 22.4 2 | 22.4 0 | 21. 56 | 21. 95 | 22. 10 | 21. 77 | 21.4 0 | 21.37 | DEPC/72.02; DEPC/72.02 | K,Y | 75,7 9 | SILuMAb- HC |
| 21.0 2 | 21.0 0 | 20.9 7 | 20.9 3 | 21.8 5 | 21.8 2 | 21. 39 | 21. 38 | 21. 19 | 21. 22 | 21.3 6 | 21.33 | DEPC/72.02; DEPC/72.02 | K,Y | 321, 323 | SILuMAb- HC |
| 20.5 4 | 20.5 5 | 20.6 4 | 20.6 6 | 20.9 4 | 20.9 6 | NaN | 20. 31 | 20. 77 | 20. 74 | 20.2 1 | 20.59 | DEPC/72.02; DEPC/72.02 | S,K | 223, 226 | SILuMAb- HC |
| 24.4 4 | 24.4 4 | 24.4 2 | 24.4 0 | 24.7 6 | 24.7 6 | 24. 38 | 24. 38 | 24. 50 | 24. 50 | 24.5 5 | 24.56 | DEPC/72.02; DEPC/72.02 | S,K | 341, 344 | SILuMAb- HC |
| 20.5 7 | 20.5 6 | NaN | 20.4 8 | 21.3 3 | 21.8 9 | 21. 52 | 21. 47 | 21. 54 | 21. 48 | 21.3 3 | 21.24 | DEPC/72.02; DEPC/72.02 | Y,K | 323, 324 | SILuMAb- HC |
| 22.3 1 | 22.3 9 | NaN | 22.1 6 | 22.8 2 | 22.8 5 | 22. 94 | 22. 98 | 22. 86 | 22. 85 | 23.3 1 | 22.50 | DEPC/72.02; DEPC/72.02 | Y,K | 323, 326 | SILuMAb- HC |

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|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------|---------------------------|-----|-------------|----------------|
| 23.3 6 | 21.8 3 | 22.9 7 | 22.9 9 | 23.4 9 | 23.4 8 | 22. 28 | 24. 34 | 23. 58 | 23. 53 | 23.3 6 | 23.33 | DEPC/72.02; DEPC/72.02 | H,K | 41,4 4 | SILuMAb- LC |
| 20.9 3 | 21.3 9 | NaN | NaN | NaN | 21.8 0 | 21. 95 | 21. 98 | NaN N | 20. 99 | 20.6 9 | 21.07 | DEPC/72.02; DEPC/72.02 | H,K | 41,4 7 | SILuMAb- LC |
| NaN | NaN | 22.8 8 | 22.8 3 | 23.3 5 | 23.2 0 | 23. 50 | NaN N | 22. 95 | 22. 94 | 22.7 4 | 22.70 | DEPC/72.02; DEPC/72.02 | K,H | 192, 194 | SILuMAb- LC |
| 22.3 3 | NaN | 22.3 2 | NaN | 22.8 1 | NaN | NaN N | 22. 13 | NaN N | 22. 41 | 22.3 7 | NaN | DEPC/72.02; DEPC/72.02 | K,S | 47,5 8 | SILuMAb- LC |
| 22.7 1 | 22.7 9 | 21.9 4 | 22.5 5 | 22.4 5 | 23.2 7 | 22. 65 | 22. 68 | 22. 90 | 22. 68 | 22.7 1 | 23.37 | DEPC/72.02; DEPC/72.02 | K,S | 162, 171 | SILuMAb- LC |
| 24.0 9 | 24.8 2 | 24.5 7 | 22.5 8 | 25.0 8 | 24.3 9 | 24. 97 | 24. 19 | 23. 86 | 24. 39 | 24.5 5 | 24.31 | DEPC/72.02; DEPC/72.02 | K,S | 177, 193 | SILuMAb- LC |
| 21.7 4 | NaN | 22.3 2 | 22.3 3 | 22.8 1 | 22.7 2 | 22. 14 | 22. 13 | NaN N | 22. 41 | 22.3 7 | 22.34 | DEPC/72.02; DEPC/72.02 | K,T | 47,5 4 | SILuMAb- LC |
| 20.6 3 | 20.6 9 | 20.5 3 | NaN | 21.8 4 | 21.2 4 | 21. 27 | 21. 98 | 21. 40 | 21. 62 | NaN | 21.07 | DEPC/72.02; DEPC/72.02 | K,Y | 44,5 1 | SILuMAb- LC |
| 21.5 2 | 21.5 2 | 21.3 0 | 21.6 5 | 22.0 5 | 21.4 5 | 22. 11 | 22. 08 | 22. 30 | 21. 61 | 21.5 0 | 22.26 | DEPC/72.02; DEPC/72.02 | K,Y | 47,5 1 | SILuMAb- LC |
| 20.8 7 | NaN | 20.6 4 | NaN | 21.2 5 | NaN | 21. 22 | 21. 19 | 21. 25 | NaN N | 21.1 0 | 21.05 | DEPC/72.02; DEPC/72.02 | K,Y | 177, 178 | SILuMAb- LC |
| 21.9 9 | 22.0 2 | 21.5 6 | NaN | NaN | 22.1 6 | NaN N | 22. 22 | NaN N | 22. 12 | 21.9 2 | 21.96 | DEPC/72.02; DEPC/72.02 | S,K | 36,4 4 | SILuMAb- LC |
| 20.0 8 | NaN | 20.5 0 | 20.4 7 | NaN | NaN | NaN N | 21. 32 | 21. 48 | 22. 41 | 22.8 6 | 22.77 | DEPC/72.02; DEPC/72.02 | S,K | 158, 162 | SILuMAb- LC |
| NaN | 21.2 7 | 21.0 6 | 20.9 9 | NaN | NaN | 21. 81 | NaN N | 21. 86 | 21. 86 | NaN | 20.52 | DEPC/72.02; DEPC/72.02 | S,K | 185, 192 | SILuMAb- LC |
| 20.5 5 | 20.4 8 | 21.3 6 | 21.2 2 | 21.1 0 | 21.0 6 | 21. 56 | 21. 55 | 21. 91 | 22. 08 | 22.3 8 | 22.72 | DEPC/72.02; DEPC/72.02 | T,K | 151, 162 | SILuMAb- LC |
| 24.4 0 | 23.7 0 | 23.3 4 | 24.0 1 | 23.8 3 | 23.8 3 | 24. 65 | 23. 90 | 24. 56 | 23. 75 | 23.6 1 | 23.58 | DEPC/72.02; DEPC/72.02 | Y,K | 32,4 4 | SILuMAb- LC |
| 23.7 0 | 23.7 0 | 23.3 4 | 23.3 5 | NaN | 23.8 3 | NaN N | 23. 21 | 23. 79 | 23. 75 | 23.6 1 | 23.58 | DEPC/72.02; DEPC/72.02 | Y,K | 38,4 4 | SILuMAb- LC |

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|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------|---|-----|-------------|----------------|
| 22.6 3 | NaN | 22.5 1 | 22.5 0 | 22.9 4 | 22.9 1 | 22. 65 | 22. 68 | 22. 93 | 22. 91 | 22.9 3 | 22.13 | DEPC/72.02; DEPC/72.02 | Y,K | 51,5 5 | SILuMAb- LC |
| 21.2 1 | 20.6 6 | 20.3 6 | 21.0 2 | 20.9 5 | NaN | 21. 10 | Na N | Na N | 21. 20 | 21.8 7 | 21.13 | DEPC/72.02; DEPC/72.02 | Y,K | 178, 192 | SILuMAb- LC |
| 21.2 1 | NaN | 23.2 2 | 23.6 2 | 24.1 9 | 23.8 8 | 23. 49 | 23. 48 | 24. 32 | 23. 77 | 23.6 0 | NaN | DEPC/72.02; DEPC/72.02 | Y,K | 183, 192 | SILuMAb- LC |
| 23.1 0 | 23.4 6 | 23.0 7 | 23.0 7 | 23.4 6 | 23.3 8 | 23. 64 | 23. 64 | 23. 62 | 23. 98 | 22.9 1 | 22.90 | DEPC/72.02; Oxidation/15. 99 | K,M | 75,8 2 | SILuMAb- HC |
| NaN | 21.1 4 | 20.9 5 | 20.9 6 | NaN | 21.3 8 | Na N | 20. 99 | 21. 03 | 21. 32 | NaN | 20.47 | DEPC/72.02; Oxidation/15. 99 | Y,M | 32,3 4 | SILuMAb- HC |
| 20.8 4 | 20.8 9 | 21.0 7 | 20.9 8 | 21.3 0 | 20.8 6 | 21. 82 | 21. 82 | 21. 94 | 21. 96 | 21.4 4 | 21.43 | DEPC/72.02; Oxidation/15. 99 | K,M | 47,4 9 | SILuMAb- LC |
| 21.5 8 | NaN | NaN | 23.8 3 | NaN | 24.3 1 | Na N | 23. 98 | 25. 57 | 23. 98 | NaN | 24.83 | DEPC/72.02; urethane-CEt histidine/134. 06 | H,H | 433, 437 | SILuMAb- HC |
| 24.1 3 | 23.3 4 | 23.9 4 | 23.0 9 | 23.6 0 | 24.4 1 | 24. 02 | 24. 85 | 24. 67 | 23. 25 | 24.0 2 | 25.53 | DEPC/72.02; urethane-CEt histidine/134. 06 | H,H | 433, 439 | SILuMAb- HC |
| NaN | NaN | 24.0 4 | 23.7 4 | 25.2 6 | NaN | Na N | Na N | Na N | 23. 78 | 25.2 4 | 24.83 | DEPC/72.02; urethane-CEt histidine/134. 06 | H,H | 437, 439 | SILuMAb- HC |
| NaN | 20.4 9 | 20.4 8 | 20.4 2 | 20.7 6 | NaN | Na N | 20. 22 | Na N | 21. 16 | 20.3 8 | 20.36 | DEPC/72.02; urethane-CEt histidine/134. 06 | K,H | 226, 228 | SILuMAb- HC |
| 22.8 4 | 22.8 1 | 22.7 4 | 22.7 5 | 23.1 5 | 24.1 7 | 23. 05 | Na N | 23. 16 | 23. 15 | 23.2 4 | 23.24 | DEPC/72.02; urethane-CEt | S,H | 430, 439 | SILuMAb- HC |

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|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|-------|-------------|------------|
| | | | | | | | | | | | | histidine/134.06 | | | |
| 22.42 | 21.68 | 21.23 | 21.90 | 22.10 | 21.96 | 21.38 | NaN | 21.15 | 21.08 | 20.68 | 20.58 | DEPC/72.02; urethane-CEt histidine/134.06;DEPC/72.02 | H,H,Y | 433,439,440 | SILuMAb-HC |
| 20.44 | 20.47 | 20.60 | NaN | 20.92 | 20.85 | 20.39 | 20.46 | 20.40 | 20.33 | 20.44 | 20.36 | Gln->pyro-Glu/-17.02;DEPC/72.02 | Q,S | 1,2 | SILuMAb-LC |
| 22.01 | 22.03 | 22.66 | 21.97 | 22.52 | 22.06 | 22.71 | 23.16 | 22.69 | 22.67 | 22.77 | 23.86 | Oxidation/15.99;DEPC/72.02 | H,K | 228,252 | SILuMAb-HC |
| 20.23 | 20.27 | 20.11 | NaN | NaN | 20.49 | 21.22 | 21.22 | 20.97 | 20.98 | 20.45 | 20.38 | Oxidation/15.99;DEPC/72.02 | M,K | 116,125 | SILuMAb-HC |
| NaN | 22.02 | 21.80 | 21.79 | 21.69 | 22.36 | 21.91 | 21.92 | 22.20 | 22.20 | 21.81 | 21.79 | Oxidation/15.99;DEPC/72.02 | M,K | 49,55 | SILuMAb-LC |
| 21.78 | 21.80 | 21.96 | 21.98 | 22.15 | 22.24 | 22.23 | 21.96 | 22.42 | 22.02 | 21.98 | 21.61 | Oxidation/15.99;DEPC/72.02 | M,Y | 49,51 | SILuMAb-LC |
| 21.21 | 21.21 | 20.86 | NaN | NaN | 20.69 | 22.10 | 22.13 | 21.77 | NaN | NaN | 21.19 | Oxidation/15.99;DEPC/72.02 | W,K | 37,44 | SILuMAb-LC |
| 22.90 | 22.78 | 21.15 | 22.66 | 21.24 | 21.24 | 21.18 | 21.11 | 21.19 | 21.19 | 20.80 | 20.81 | Oxidation/15.99;DEPC/72.02;urethane-CEt histidine/134.06 | M,H,H | 432,433,439 | SILuMAb-HC |

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|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|-----|---------|------------|
| 20.08 | 20.18 | 20.76 | 20.02 | 20.56 | 21.04 | 20.76 | 20.92 | 20.98 | 20.96 | 20.97 | 20.93 | urethane-CEt histidine/134.06;DEPC/72.02 | H,K | 228,250 | SILuMAb-HC |
| 21.36 | 21.39 | NaN | NaN | NaN | 21.84 | 22.39 | 22.40 | 22.25 | 22.25 | 22.34 | 20.04 | urethane-CEt histidine/134.06;DEPC/72.02 | H,K | 228,252 | SILuMAb-HC |
| 20.44 | 20.42 | 20.70 | 20.22 | 21.23 | 20.76 | 21.17 | 21.22 | NaN | 21.05 | NaN | 21.13 | urethane-CEt histidine/134.06;DEPC/72.02 | H,K | 314,321 | SILuMAb-HC |
| 20.24 | 20.19 | NaN | NaN | 20.76 | NaN | 21.62 | 21.59 | 21.29 | 21.27 | 21.24 | 21.15 | urethane-CEt histidine/134.06;DEPC/72.02 | H,K | 314,324 | SILuMAb-HC |
| 22.84 | NaN | 22.74 | 22.75 | 23.15 | 24.50 | 23.04 | 23.06 | NaN | 24.11 | 23.24 | 22.47 | urethane-CEt histidine/134.06;DEPC/72.02 | H,T | 437,441 | SILuMAb-HC |
| 23.78 | 24.42 | 22.80 | 22.63 | 24.17 | 23.28 | NaN | 23.62 | NaN | 24.30 | 24.17 | 22.87 | urethane-CEt histidine/134.06;DEPC/72.02 | H,Y | 437,440 | SILuMAb-HC |
| NaN | 23.88 | 24.26 | 23.74 | 24.21 | 24.20 | NaN | 23.72 | 23.80 | 23.78 | 23.76 | 23.75 | urethane-CEt histidine/134.06;DEPC/72.02 | H,Y | 439,440 | SILuMAb-HC |
| 22.04 | 22.01 | 21.28 | 21.17 | 21.94 | 21.99 | 21.41 | 21.45 | 21.90 | 21.88 | 21.50 | 21.50 | urethane-CEt histidine/134.06;DEPC/72.02 | H,K | 41,44 | SILuMAb-LC |

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|-----------|-----------|-----------|-----|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------|--|-----------|---------------------|----------------|
| 22.0 3 | 22.0 7 | 21.3 9 | NaN | 22.3 3 | 21.2 1 | 22. 55 | 22. 56 | 22. 18 | 22. 20 | 21.9 0 | 21.88 | urethane-CEt histidine/134. 06;DEPC/72. 02;DEPC/72. 02 | H,K, T | 228, 250, 254 | SILuMAb- HC |
|-----------|-----------|-----------|-----|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------|--|-----------|---------------------|----------------|

Table S3: Percentage DEPC incorporation at Cys for assessing the intactness of disulfide bonds in long term stored (S_2yr, S_1yr) and fresh SILuMAb- each with 3 replicates.

| SILuMAb -HC | Cys position | S_2yr (1) | S_2yr (2) | S_2yr (3) | S_2yr (4) | S_1yr (1) | S_1yr (2) | S_1yr (3) | S_1yr (4) | S_0yr (1) | S_0yr (2) | S_0yr (3) | S_0yr (4) |
|----------------|-----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| | 22 | 1.46 | | | 1.4 | 2.14 | | | | | | | |
| | 95 | 2.29 | 2.55 | 2.41 | 2.09 | 2.94 | 2.95 | | | | | | |
| | 148 | 15 | 15.2 | 14.7 | 14.6 | 16.6 | 16.9 | 18.4 | 19.1 | | 33.9 | | |
| | 230 | | 14.7 | 13.9 | 14.1 | | 16.5 | | | | | | |
| | 233 | 14.7 | 15.2 | 13.9 | 14 | 18.1 | 16.5 | | 20.6 | | | | |
| | 265 | 8.55 | 4.39 | 4.55 | 4.73 | 7.33 | 4.96 | 6.3 | 10.1 | | | | |
| | 429 | | | | 23.7 | 25.9 | | 28.2 | 26.2 | | | | |
| SILuMAb -LC | 199 | 30.6 | 21.2 | 21.4 | 16.2 | 21.8 | 16.3 | 64.4 | 66.8 | | | | |

Supporting method 1: Peptide cleanup using C18 stagetips:

In brief, two layers of C18 solid phase extraction material were manually tightly filled into 100 μ L pipette tips. The C18 material was activated with 100 μ L methanol and then 100 μ L 0.1% FA in 80% ACN. The tips were centrifuged after each step at 2000 x g for 2 min or until the solutions completely passed through the C18 phase. For equilibration, the tips were washed with 100 μ L 0.1% FA in water (twice), and liquid was drained by centrifugation. Next, 200 μ L of tryptic digest was added at a time to the prepared tips and centrifuged until the whole sample was loaded. StageTips were washed twice with 100 μ L 0.1% FA water. The C18 disk-bound tryptic peptides were eluted with 100 μ L of 0.1% FA in 80% ACN.

Supporting method 2: Detailed method for LC-MS based tryptic peptide analysis

Mobile phase A consisted of 0.1% FA in water, mobile phase B of 0.1% FA in ACN. Column oven temperature was set to 40 °C and flow rate was 12 µL/min. The gradient (total time 45 min) was as follows: 2 min 5% B, then within 30 min up to 40% B, then up to 90% B within 1 min, keeping it for 4 min at 90% B, and finally down to 5% B within 1 min. The column is re-equilibrated for 7 min at 5% B. For the ESI source, end plate offset was 500 V, capillary offset 4500 V, nebulizer gas flow 0.7 bar, dry gas flow 6L/min, and dry temp 200 °C. Auto MS/MS scan mode was applied with the following parameters: cycle time 2s, threshold per 1000, sum 500 cts, fixed MS/MS acquisition 0.68 s. For each sample, two technical replicates were recorded.

Supporting method 3: Detailed method for LC-MS based intact protein mass analysis

Mobile phase A consisted of 0.1% FA in water, mobile phase B of 0.1% FA in ACN. Column oven temperature was set to 80 °C and flow rate was 0.35 mL/min. The gradient was as follows: 20% B at 4 min, 40% B at 5min, 70% B at 10 min, 90% B at 11 min, 20% B at 12 min, 20% B at 15 min. For the ESI source, end plate offset was 500 V, capillary offset 4500 V, nebulizer gas flow 3 bar, dry gas flow 12 L/min, and dry temp. 300 °C and in-source CID 120 eV

Supporting method 4: Detailed data analysis method for quantification of peptides from LC-MS/MS data

Data analysis was performed in Byos software from Protein metrics. For identification of peptides, the modification list included carbamidomethylation (57 Da) as fixed modification in most experiments. Only for the long-storage experiment, carbamidomethylation was set to variable modification. Otherwise, common variable modifications included pyroglutamate formation at the protein N terminus (-17.02 Da), oxidation (15.99 Da) at Met, carbethoxy modification with DEPC reagent (72.02 Da) at His, Lys, Cys, Ser, Thr, and Tyr. Neutral losses of DEPC from Lys and His (46.04 Da) or neutral loss from Ser and Thr (90.03 Da), were set as rare variable modifications. To allow maximum identification of DEPC modified peptides, other rare modifications considered were urethane carbethoxy histidine (134.06 Da), formyl carbethoxy histidine (162.05 Da) and di- carbethoxy histidine (146.06 Da). A total of four modifications (two of common and two of rare modifications) were allowed per peptide. Results were filtered to only include peptides with 2-7 charges, a peptide score of >150, delta modification score >10 and extracted ion chromatogram (XIC) area >3e5.