|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **potential**  | **Peptide Sequence** | **Start** | **Stop** | **Modifications** | **Localization** | **Ascore** | **Mascot:** | **Filter** |
| **acetylation site** |  **probability (%)** | **Score** |
| K7 | FTW**k**ELIQLGSPSK | 4 | 17 | K4 Acetyl | 97 | 19.84 | 65.08 | CID |
| K7 | FTW**k**ELIqLGSPSK | 4 | 17 | K4 Acetyl | 100 | 33.18 | 47.28 | CID |
| K17 | ELIQLGSPS**k** | 8 | 17 | K10 Acetyl | 98 | 17.01 | 42.73 | CID |
| K137 | **k**ALkIFK | 137 | 143 | K1 Acetyl, K4 Acetyl | 100 | 151.71, 83.60 | 39.11 | CID |
| K272 | NVc**k**LASNYK | 269 | 278 | K4 Acetyl | 100 | 73.31 | 48.92 | CID |
| K272 | NVc**k**LASnYK | 269 | 278 | K4 Acetyl | 100 | 32.69 | 28.55 | CID |
| K287 | KPDAQTIV**k**NDcLLDFLDcGK | 279 | 299 | K9 Acetyl | 100 | 68.47 | 69.06 | CID |
| K287 | KPDAQTIV**k**NDcLLDFLDcGK | 279 | 299 | K9 Acetyl | 100 | 27.94 | 59.57 | CID |
| K351 | V**k**QSDYDR | 350 | 357 | K2 Acetyl | 100 | 39.76 | 39.79 | HCD/ETD |
| K373 | TADLAE**k**LFK | 367 | 376 | K7 Acetyl | 100 | 53.53 | 48.55 | CID |
| K498 | SYYPLT**k**LSmTITNFDIIDLQK | 492 | 513 | K7 Acetyl | 97 | 24.32 | 36.45 | CID |
| K532, K537 | SSAG**k**EDEE**k**TTSSK | 528 | 542 | K5 Acetyl, K10 Acetyl | 89, 99 | 16.66, 26.02 | 42.61 | CID |
| K542, K546 | TTSS**k**ADE**k**TPK | 538 | 549 | K5 Acetyl, K9 Acetyl | 91, 100 | 17.01, 30.46 | 40.05 | CID |
| K603, K615 | **k**RPNSQHTATPQ**k**K | 603 | 616 | K1 Acetyl, K13 Acetyl | 95, 63 | 17.39, 8.22 | 13.27 | HCD/ETD |

**Table S3A. Mass spectrometry detected Eco1-mediated Polη acetylation sites *in vitro***

**(selected, with localization probability ≥ 90% or Mascot scores ≥ 30)**

**Table S3B. Mass spectrometry detected break specific Polη acetylation sites *in vivo***

**(selected, with localization probability ≥ 90% or Mascot scores ≥ 30)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| **potential acetylation site** | **Peptide Sequence** | **Start AA** | **Stop AA** | **Modifications** | **Localization Probability (%)** | **Ascore** | **Mascot:Score** |
| K279 | **k**PDAqTIVK | 279 | 287 | K1 Acetyl | 100 | 35.23 | 12.03 |
| K287 | KPDAQTIV**k**nDcLLDFLDcGK | 279 | 299 | K9 Acetyl | 100 | 57.60 | 31.97 |
| K399 | SmMSN**k**nLR | 394 | 402 | K6 Acetyl | 100 | 32.28 | 28.93 |
| K436 | IQDLEQEYn**k**IVIPR | 427 | 441 | K10 Acetyl | 50 | 0.00 | 31.24 |
| K491 | n**k**SYYPLTK | 490 | 498 | K2 Acetyl | 98 | 23.98 | 21.36 |