

## Supplementary Table S2

Statistics of underlying LC-MS runs together with the corresponding triggered MS-Scans and PEAKS database searches of HeLa lysate digested with trypsin, LysC and neprosin.

Sample	HeLa + Trypsin			HeLa + LysC			HeLa + Neprosin		
Cleavage	None	P1: K, R P1': Any	P1: K, R P1': Any	None	P1: K P1': Any	P1: K P1': Any	None	P1: P, A P1': not P And P1: D with P1': P	P1: P, A P1': not P And P1: D with P1': P
Nonspecific cleavage	Both ends	One end	None	Both ends	One end	None	Both ends	One end	None
Missed cleavages	100	3	2	100	3	2	100	7	7
Usage	-	Proteome analysis	PTM analysis	-	Proteome analysis	PTM analysis	-	Proteome analysis	PTM analysis
Database	Homo sapiens	Homo sapiens	Homo sapiens	Homo sapiens	Homo sapiens	Homo sapiens	Homo sapiens	Homo sapiens	Homo sapiens
TIC	4.3 * 10 <sup>9</sup>			2.6 * 10 <sup>9</sup>			3.7 * 10 <sup>9</sup>		
# MS scans	13014			11795			10758		
# MS/MS scans	60859			48219			63308		
# CID spectra	51877			34304			39317		
# ETD spectra	8982			13915			23991		
# PSMs	27798	31388	26395	19802	22336	17248	11538	12975	10504
Total conversion rate	46 %	52 %	44 %	41 %	46 %	36 %	18 %	20 %	17 %
CID conversion rate	46 %	52 %	43 %	38 %	44 %	34 %	15 %	17 %	13 %
ETD conversion rate	50 %	54 %	48 %	50 %	55 %	44 %	24 %	28 %	23 %
# peptide sequences	22298	25240	20814	14988	16955	12628	7879	8765	6997
# unique cleavages	39167	-	-	25224	-	-	10713	-	-
# protein groups	3214	3505	3433	2510	2826	2715	1617	1729	1644