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### Fast and Simple Protocols for Mass Spectrometry-Based Proteomics of Small Fresh Frozen Uterine Tissue Sections

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**Fast and simple protocols for mass spectrometry-based proteomics  
of small fresh frozen uterine tissue sections**

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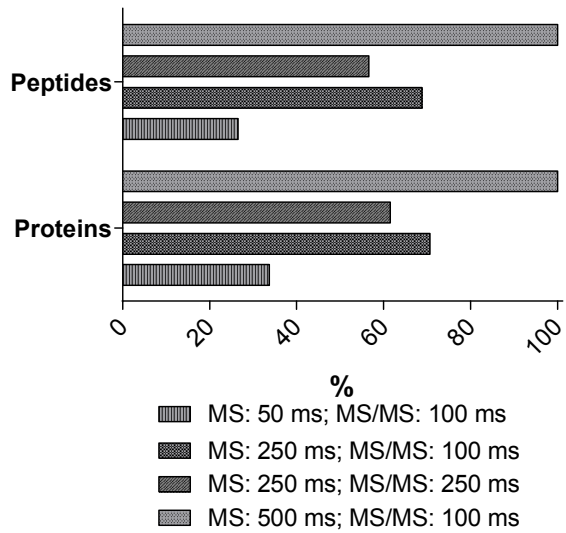
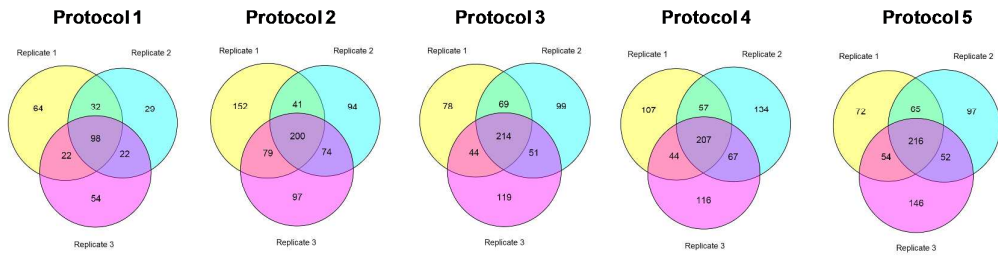


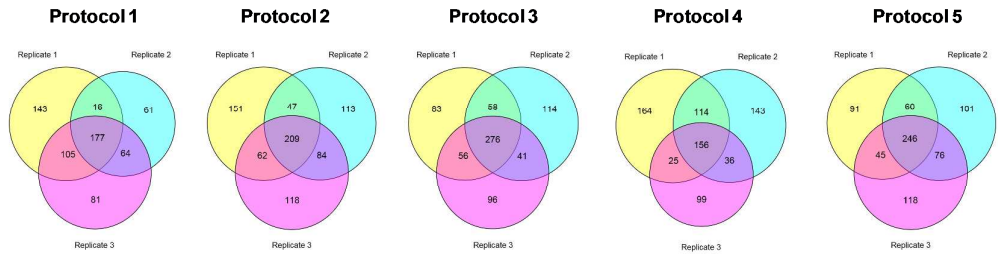
Figure S-1. Influence of the instrumental parameters on number of identified proteins and peptides. Results are displayed for optimization of instrumental parameters for both TOF-MS and TOF-MS/MS accumulation time. Data were recorded in intensity dependent acquisition (IDA) mode with the following parameters: TOF-MS ( $m/z$ : 400-1250; threshold 100 cps; top ions: 30 with charge from +2 to +4; exclusion time: 30 s); TOF-MS/MS ( $m/z$ : 200-1800). Other parameters regarding instrumental settings were as described in Experimental section.

**10 μm tissue**



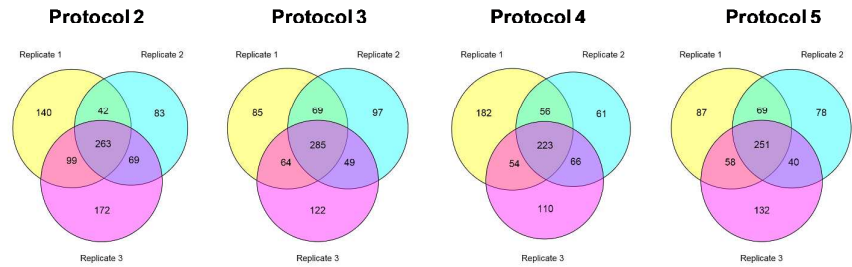
a)

**16 μm tissue**

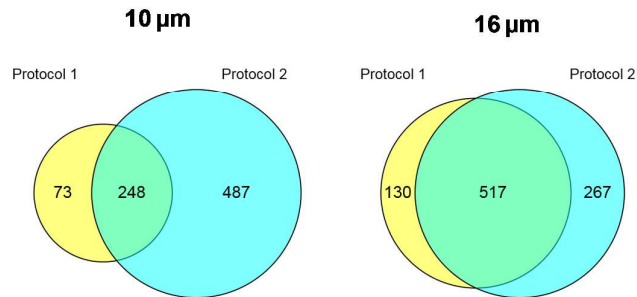


b)

**20 μm tissue**

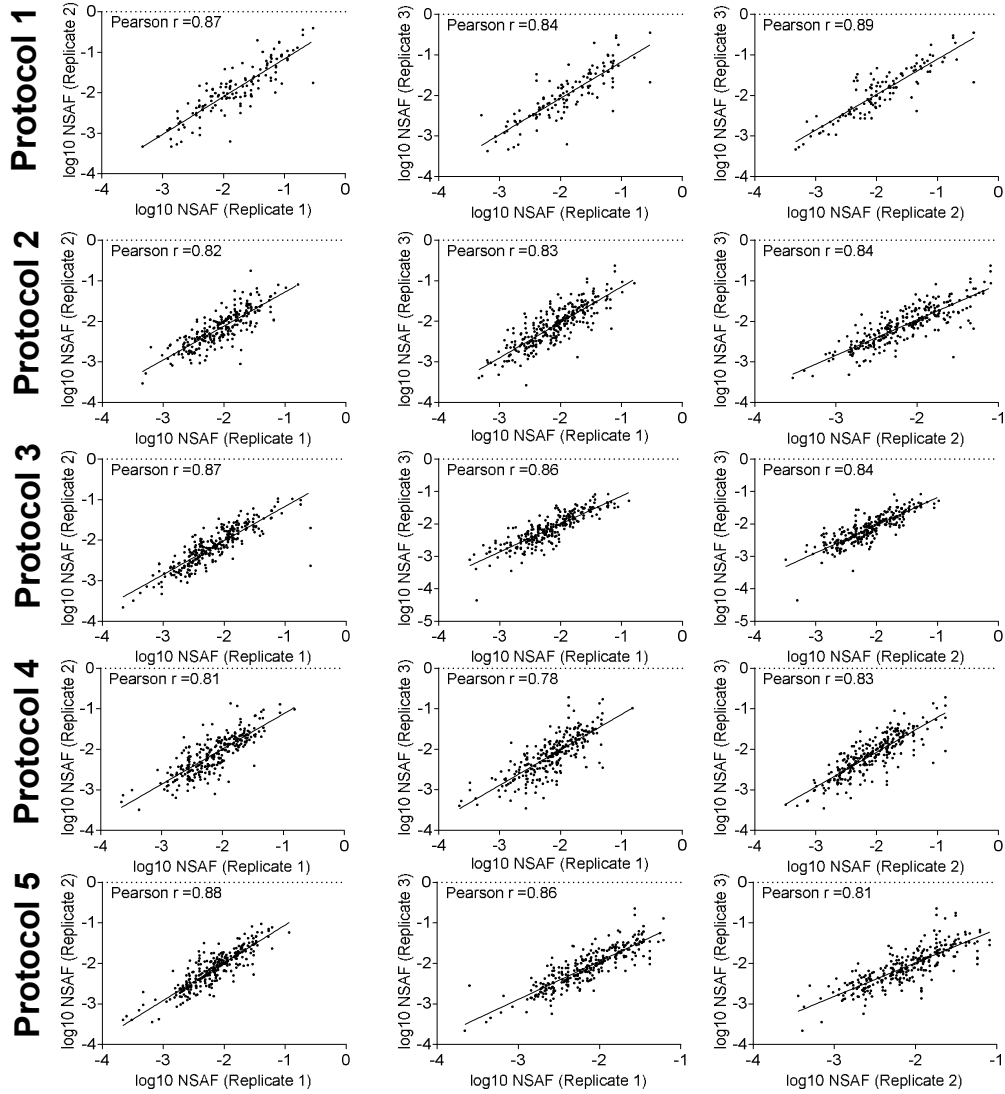


c)

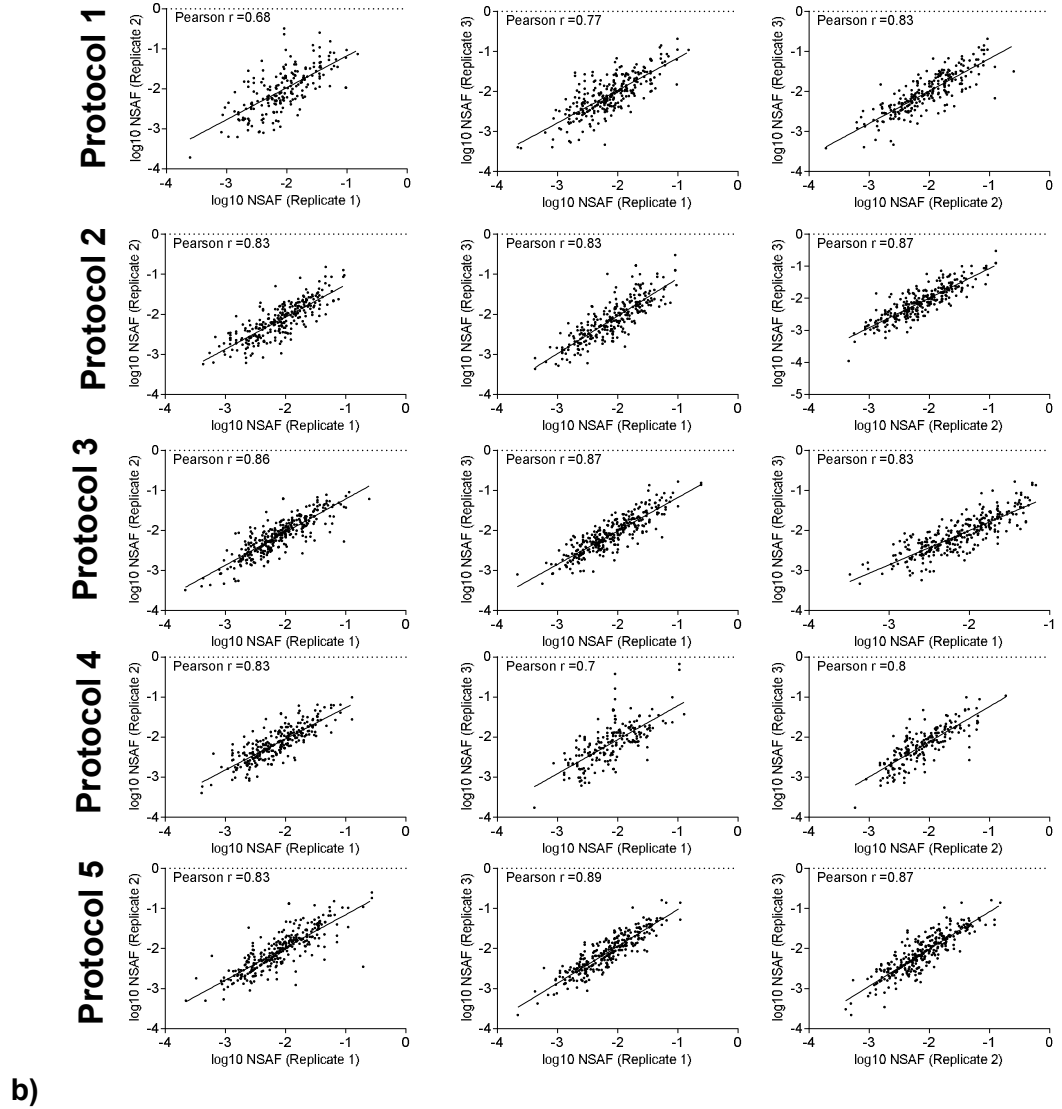


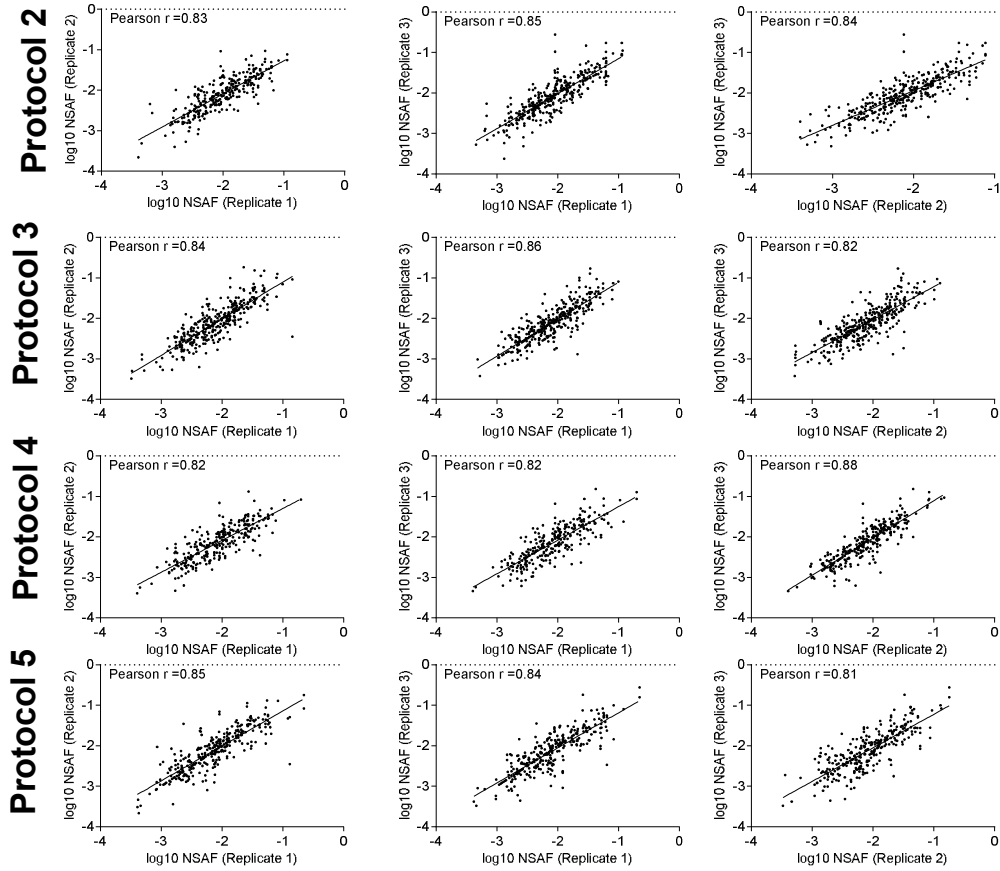
d)

Figure S-2. Venn diagrams showing qualitative reproducibility of the proteins between 3 biological replicates for 10, 16 and 20 μm tissue (a-c); shared proteins for 10 μm and 16 μm thick tissue identified from 3 replicates for Protocol 1 and 2 (d).



a)





c)

Figure S-3. Quantitative reproducibility of the proteins between 3 biological replicates of FF human uterus tissue. Results are shown as correlation of the NSAF values of the identified proteins for (a) 10, (b) 16 and (c) 20  $\mu\text{m}$  thick FF human uterus tissue.



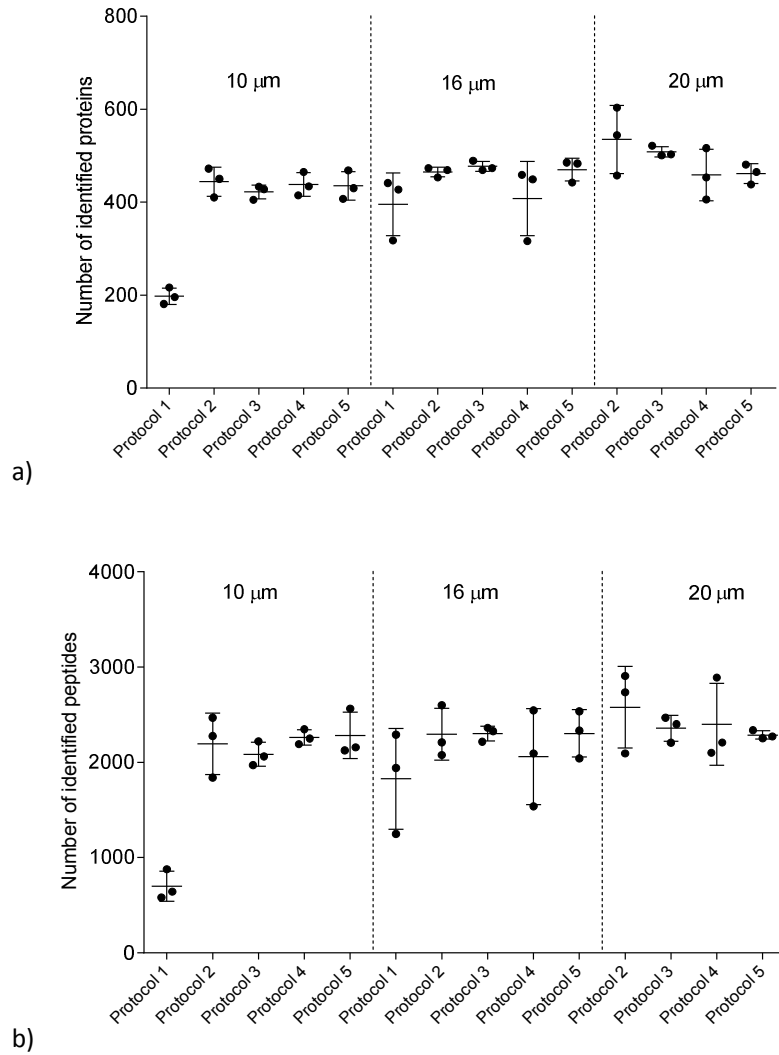


Figure S-4. Number of identified proteins (a) and peptides (b) using different extraction and digestion conditions. Proteins and peptides were identified at 1% FDR in 10, 16 and 20  $\mu\text{m}$  thick tissue after protein extraction and digestion using Protocols 1 to 5. Results are shown as mean $\pm$ SD.

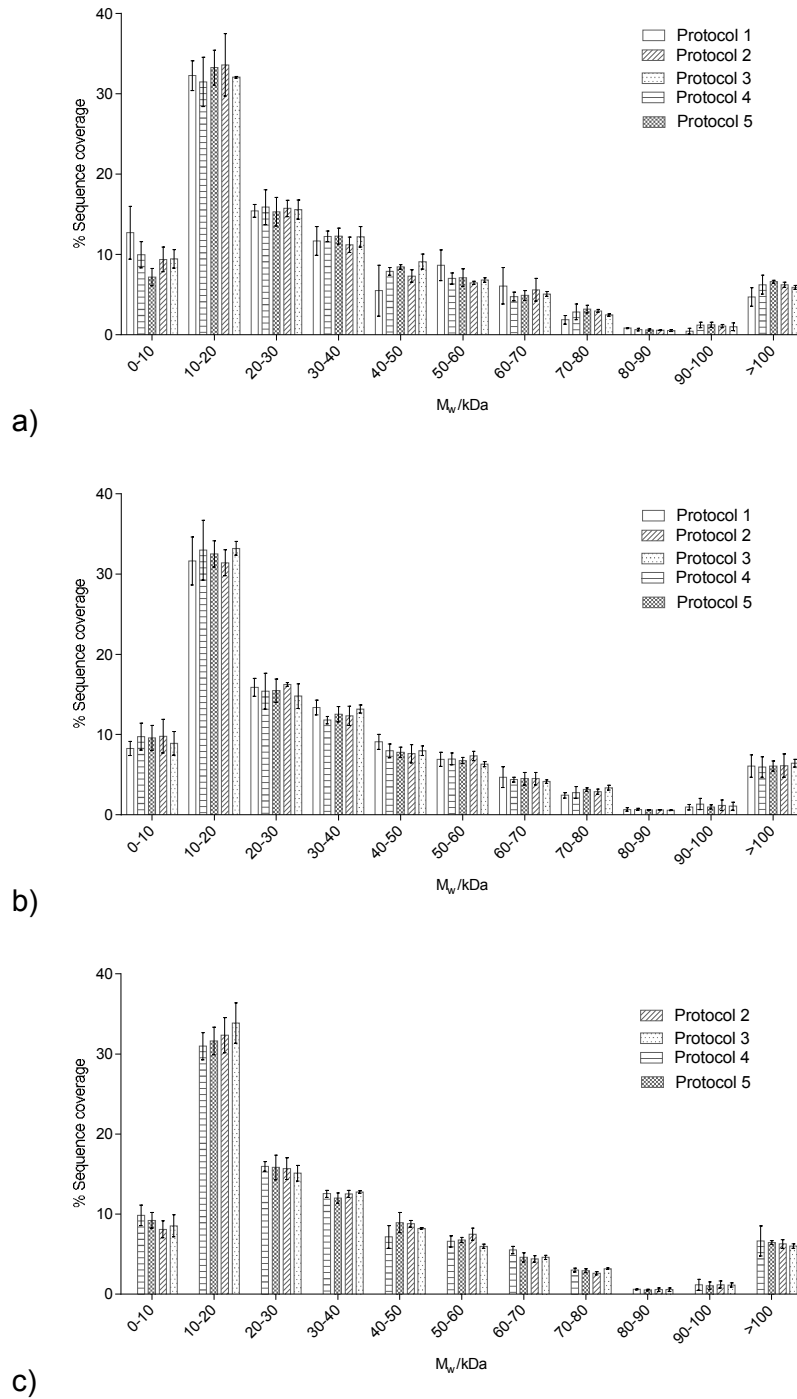


Figure S-5. Results from the study evaluated for the sequence coverage of the proteins extracted from 3 biological replicates distributed according to their  $M_w$ : a) 10  $\mu\text{m}$ , b) 16  $\mu\text{m}$  and c) 20  $\mu\text{m}$  thick FF human uterus tissue. Results are shown as mean  $\pm$  SD.

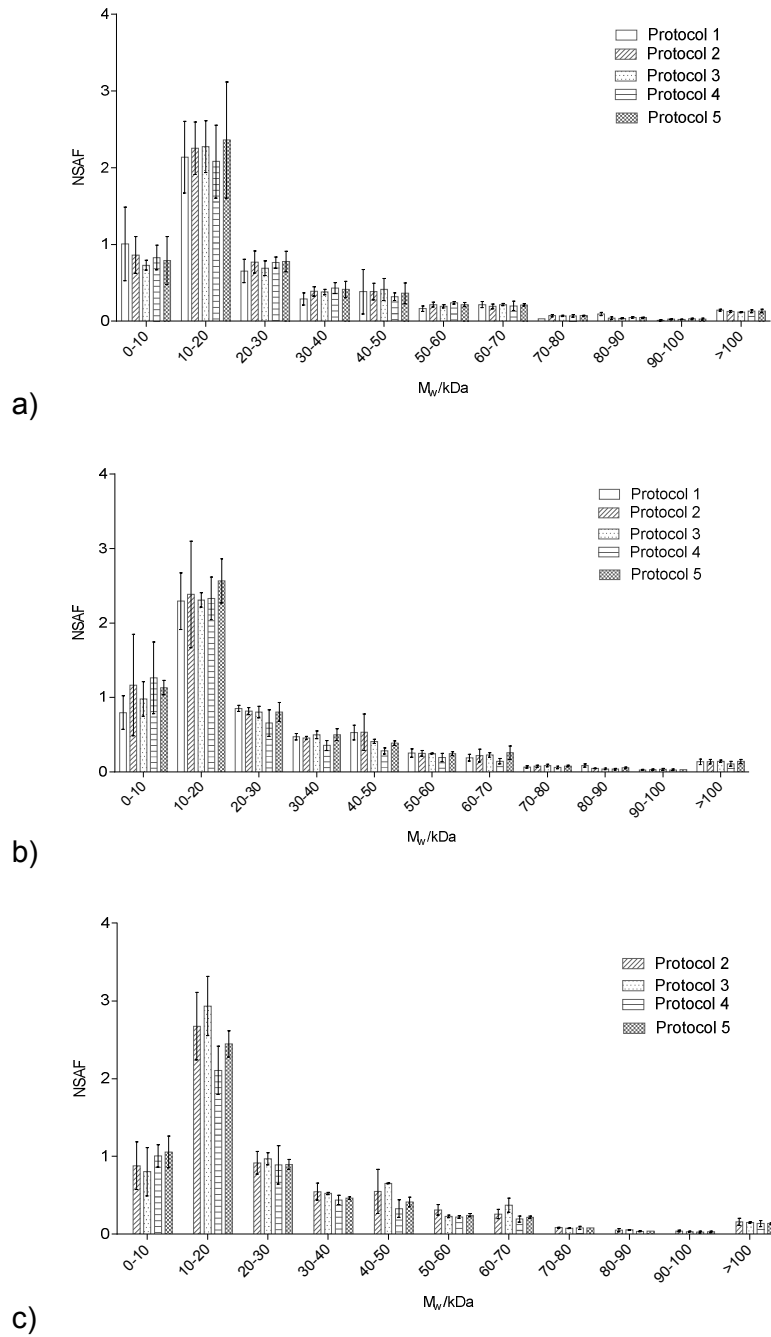


Figure S-6. NSAF values of the proteins extracted from 3 biological replicates distributed according to their  $M_w$ : a) 10  $\mu\text{m}$ , b) 16  $\mu\text{m}$  and c) 20  $\mu\text{m}$  thick FF human uterus tissue. Results are shown as mean $\pm$ SD.