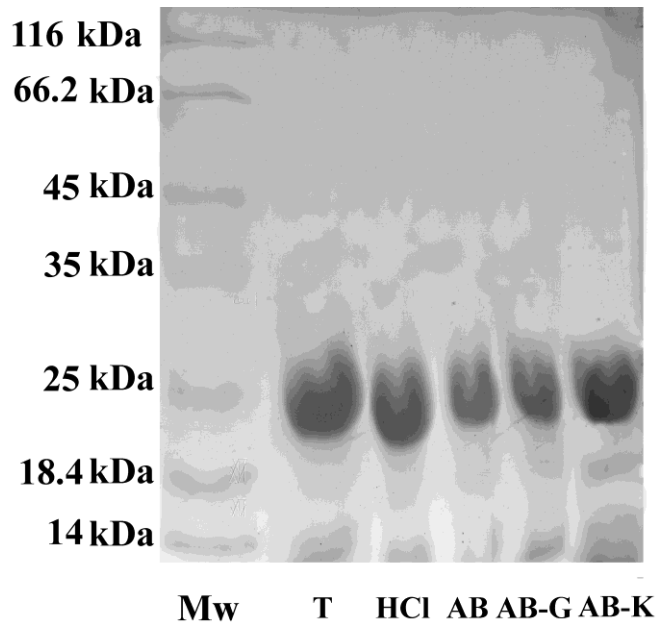


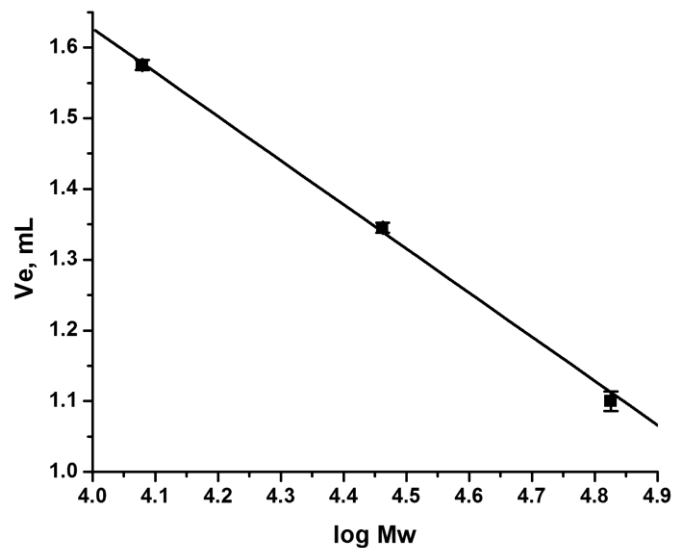
Supplementary material for the article:

Rašković, B.; Vatić, S.; Anđelković, B.; Blagojević, V.; Polović, N. Optimizing Storage Conditions to Prevent Cold Denaturation of Trypsin for Sequencing and to Prolong Its Shelf Life. *Biochemical Engineering Journal* **2016**, *105*, 168–176.

<https://doi.org/10.1016/j.bej.2015.09.018>



Supplementary Figure S1. SDS PAGE analysis of the untreated trypsin (T) and trypsin samples after 7 freeze-thaw cycles. A) Trypsin dissolved in 1 mM HCl and B) Trypsin dissolved in 100 mM ammonium-bicarbonate (AB) with 0.3 M glycerol (AB-G) and 0.1 M lysine (AB-K).



Supplementary Figure S2. Calibration plot used to determine molecular weight of trypsin in gel filtration analysis. Bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome c (12.4 kDa) were used as molecular weight markers.

Table S1. BSA-specific peptides identified in the samples digested with differently treated trypsin (Untreated, HCl, AB-G and AB-K).

<b>Trypsin sample</b>	<b>Start-end</b>	<b>Peptide</b>
<b>HCl; AB-K</b>	29-36	SEIAHRFK
<b>AB-K</b>	66-88	LVNELTEFAKTCVADESHAGCEK
<b>Untreated; HCl</b>	76-88	TCVADESHAGCEK
<b>AB-K</b>	89-100	SLHTLFGDELCK
<b>Untreated; AB-G; AB-K</b>	101–105	VASLR
<b>Untreated; HCl; AB-K</b>	106-117	ETYGDMADCCEK
<b>Untreated</b>	118-122	QEPER
<b>Untreated; HCl; AB-G; AB-K</b>	131–138	DDSPDLPK
<b>Untreated</b>	139–156	LKPDPNTLCDEFKADEKK
<b>AB-G; AB-K</b>	152-160	ADEKKFWGK
<b>Untreated; AB-G; AB-K</b>	157–160	FWGK
<b>Untreated</b>	161–167	YLYEIAR
<b>Untreated; AB-G; AB-K</b>	198–204	GACLLPK
<b>AB-G</b>	219-222	QRLR
<b>Untreated; AB-G; AB-K</b>	223–228	CASIQK
<b>Untreated; AB-G; AB-K</b>	229–232	FGER
<b>Untreated; AB-G</b>	236–241	AWSVAR
<b>AB-G</b>	242–245	LSQK
<b>Untreated; AB-G; AB-K</b>	246-248	FPK

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<b>AB-G; AB-K</b>	246-266	FPKAEFVEVTKLVTDLTKVHK
<b>Untreated; HCl; AB-G;</b>	249-256	AEFVEVTK
<b>AB-K</b>		
<b>Untreated; AB-G; AB-K</b>	257-263	LVTDLTK
<b>Untreated; AB-K</b>	264-266	VHK
<b>Untreated</b>	264-280	VHKECCHGDLLECADDR
<b>AB-G</b>	281-285	ADLAK
<b>AB-G</b>	281-297	ADLAKYICDNQDTISSK
<b>HCl; AB-K</b>	286-297	YICDNQDTISSK
<b>HCl; AB-G; AB-K</b>	286-318	YICDNQDTISSKLECCDKPLLEKSHCIAEVEK
<b>HCl</b>	310-318	SHCIAEVEK
<b>HCl; AB-G; AB-K</b>	310-340	SHCIAEVEKDAIPENLPPLTADFAEDKDVCK
<b>Untreated; AB-G; AB-K</b>	341-346	NYQEAK
<b>Untreated</b>	361-374	HPEYAVSVLLRLAK
<b>AB-G</b>	372-386	LAKEYEATTLEECCA
<b>HCl; AB-G</b>	375-386	EYEATTLEECCA
<b>Untreated; AB-K</b>	413-436	QNCDQFEKLGEGYGFQNALIVRYTR
<b>AB-G</b>	434-436	YTR
<b>Untreated; HCl; AB-G;</b>	434-451	YTRKVPQVSTPTLVEVSR
<b>AB-K</b>		
<b>Untreated; AB-G; AB-K</b>	452-455	SLGK
<b>Untreated; AB-G</b>	456-459	VGTR
<b>HCl; AB-G; AB-K</b>	469-482	MPCTEDYLSLILNR

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<b>HCl</b>	483-489	LCVLHEK
<b>Untreated; AB-G; AB-K</b>	490-495	TPVSEK
<b>Untreated</b>	496-498	VTK
<b>Untreated; AB-G; AB-K</b>	524-528	AFDEK
<b>Untreated</b>	524-547	AFDEKLFTFHADICTLPDTEKQIK
<b>Untreated; HCl; AB-G; AB-K</b>	529-548	LFTFHADICTLPDTEKQIKK
<b>Untreated; AB-K</b>	545-548	QIKK
<b>Untreated</b>	545-557	QIKKQTALVELLK
<b>HCl</b>	548-557	KQTALVELLK
<b>AB-K</b>	558-561	HKPK
<b>Untreated; AB-G; AB-K</b>	562-568	ATEEQLK
<b>AB-G; AB-K</b>	569-580	TVMENFVAFVDK
<b>Untreated; AB-G; AB-K</b>	581-597	CCAADDKEACFAVEGPK
<b>Untreated; AB-G</b>	588-597	EACFAVEGPK
<b>Untreated; HCl; AB-G; AB-K</b>	598-607	LVVSTQTAL

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Table S2. Trypsin autolytic peptides found in all tested samples (Untreated, HCl, AB-G and AB-K). Peptides marked with asterisk were missing in the trypsin AB-K sample.

<b>Start-end</b>	<b>Monoisotopic mass (m/z)</b>	<b>Peptide</b>
<b>113-114</b>	260.197	LK
<b>160-162</b>	363.206	CLK
<b>241-246*</b>	633.320	QTIASN
<b>67-72</b>	659.383	SGIQVR
<b>115-122</b>	805.416	SAASLNSR
<b>224-231*</b>	906.504	NKPGVYTK
<b>163-172</b>	1020.503	APILSDSSCK
<b>232-240</b>	1111.560	VCNYVSWIK
<b>149-159</b>	1153.574	SSGTSYPDVLK
<b>194-209</b>	1495.615	DSCQGDSGGPVVCSGK
<b>173-193</b>	2193.994	SAYPGQITSNMFCAGYLEGGK
<b>123-148*</b>	2552.248	VASISLPTSCASAGTQCLISGWGNTK