

**Supplement to:** T. M. Grunina, A. V. Demidenko, A. M. Lyaschuk, M. S. Poponova, Z. M. Galushkina, L. A. Soboleva, S. A. Cherepushkin, N. B. Polyakov, D. A. Grumov, A. I. Solovyev, V. G. Zhukhovitsky, I. S. Boksha, M. E. Subbotina, A. V. Gromov, V. G. Lunin, and A. S. Karyagina, Recombinant Human Erythropoietin with Additional Processable Protein Domains: Purification of Protein Synthesized in *Escherichia coli* Heterologous Expression System (ISSN 0006-2979, *Biochemistry (Moscow)*, 2017, Vol. 82, No. 11, pp. 1285-1294)

**Table S1.** Peptides from 6His-s-tag-EPO protein produced with in-gel trypsinolysis in the presence of DTT and iodoacetamide and identified by MALDI mass-spectrometry

No.	Peptide name	m/z measured	Mr calc.	z	$\Delta$ m/z [ppm]	Sequence*	Modifications	Range
1	T1	2145.0567	2144.0017	1	22.25	-.MHHHHHHSSGLVPRGSGMK.E	oxidation: 1	1-19
2	T1	2145.0567	2144.0017	1	22.25	-.MHHHHHHSSGLVPRGSGMK.E	oxidation: 18	1-19
3	T2	1022.5121	1021.5193	1	-14.10	K.ETAALKFER.Q		20-28
4	T3	763.4468	762.3694	1	91.88	R.LICDSR.V	carbamidomethylation: 3	55-60
5	T4	736.4902	735.4167	1	89.97	R.YLLEAK.E		65-70
6	T5	2803.1903	2802.2436	1	-21.61	K.EAENITGCAEHCSLNENITVPDTK.V	carbamidomethylation: 9, 13	71-95
7	T6	927.4832	926.4650	1	11.73	K.VNFWYAWK.R		96-102
8	T7	1083.5305	1082.5661	1	-39.58	K.VNFWYAWKR.M		96-103
9	T8	2542.2284	2541.3261	1	-41.29	R.MEVGQQAVEVWQGLALLSEAVLR.G	oxidation: 1	104-126
10	T9	2359.1831	2358.2332	1	-24.30	R.GQALLVNSSQPWEPLQLHVDK.A		127-147
11	T10	2942.3611	2941.5774	1	-75.98	R.GQALLVNSSQPWEPLQLHVDKAVSGLR.S		127-153
12	T11	803.5392	802.4913	1	50.60	R.SLTLLR.A		154-160
13	T12	2034.1281	2033.0906	1	14.90	R.ALGAQKEAISPPDAASAAPLR.T		161-181
14	T13	1465.7911	1464.7573	1	18.12	K.EAISPPDAASAAPLR.T		167-181
15	T14	924.4911	923.4712	1	13.61	R.TITADTFR.K		182-189
16	T15	1052.5648	1051.5662	1	-8.25	R.TITADTFRK.L		182-190
17	T16	898.4904	897.4709	1	13.62	R.VYSNFLR.G		194-200
18	T17	1083.5305	1082.5873	1	-59.08	R.VYSNFLRGK.L		194-202
19	T18	1210.5994	1209.6176	1	-21.04	K.LKLYTGEACR.T	carbamidomethylation: 9	203-212
20	T19	969.4527	968.4386	1	7.08	K.LYTGEACR.T	carbamidomethylation: 7	205-212

\* Amino acids located before and after the dot are not in the peptide – these amino acids flank the identified sequence.

**Table S2.** Peptides from 6His-s-tag-EPO protein produced with in-gel trypsinolysis without addition of DTT and iodoacetamide and identified by MALDI mass-spectrometry

No.	Peptide name	m/z measured	Mr calc.	z	$\Delta$ m/z [ppm]	Number of missed cleavages	Sequence*	Modifications	Range
1	T20	1668.7895	1667.7964	1	-8.51	0	-.MHHHHHHSSGLVPR.G		1-14
2	T20	1684.8059	1683.7913	1	4.36	0	-.MHHHHHHSSGLVPR.G	oxidation: 1	1-14
3	T1	2128.9941	2128.0068	1	-9.37	1	-.MHHHHHHSSGLVPRGSGMK.E		1-19
4	T1	2145.0341	2144.0017	1	11.72	1	-.MHHHHHHSSGLVPRGSGMK.E	oxidation: 18	1-19
5	T1	2145.0341	2144.0017	1	11.72	1	-.MHHHHHHSSGLVPRGSGMK.E	oxidation: 1	1-19
6	T21	1498.7268	1497.7246	1	-3.39	2	R.GSGMKETAAAKFER.Q	oxidation: 4	15-28
7	T22	3219.1965	3218.3881	1	-61.76	2	K.FERQHMDSPDLGTDDDDKA-MADIGSAPPR.L	oxidation: 6, 20	26-54
8	T23	2787.1233	2786.1760	1	-21.49	1	R.QHMDSPDLGTDDDDKA-MADIGSAPPR.L	oxidation: 3, 17	29-54
9	<b>T24-S-S</b>	<b>4515.0148</b>	<b>4513.9883</b>	<b>1</b>	<b>4.26</b>	<b>0</b>	-. peptides: QHMDSPDLGTDDDDKA-MADIGSAPPR.LICFDSR(EPO S-S ...,29-61) 3: oxidation:57(29)<->LYTGEACR(EPO S-S ...,206-213):212(7) .-		29-61
10	<b>T24-S-S</b>	<b>4515.0148</b>	<b>4513.9883</b>	<b>1</b>	<b>4.26</b>	<b>0</b>	-. peptides: QHMDSPDLGTDDDDKA-MADIGSAPPR.LICFDSR(EPO S-S ...,29-61) 17: oxidation:57(29)<->LYTGEACR(EPO S-S ...,206-213):212(7) .-		29-61
11	T25	1101.5251	1100.5284	1	-9.63	0	K.AMADIGSAPPR.L	oxidation: 2	44-54
12	<b>T3+[191-217]</b>	<b>4042.127</b>	<b>4042.127</b>	<b>1</b>	<b>0.11</b>		<b>LICFDSR 3: Crosslink12To[KLFRVYS-NFLRGKLYTGEACRTGDR]</b>		55-61
13	<b>T19+[55-65]</b>	<b>2259.933</b>	<b>2260.121</b>	<b>1</b>	<b>-83.369</b>		<b>LICFDSRVLER 3: Crosslink1To[LYTGEACR]</b>		55-65
14	T4	736.4445	735.4167	1	27.90	0	R.YLLEAK.E		66-71
15	<b>T26[66-96]S-S</b>	<b>3404.289</b>	<b>3404.598</b>	<b>1</b>	<b>-90.853</b>	<b>1</b>	<b>YLLEAKEAENITGCAEHCSLNEN-ITVPDTK 15: Oxidized (SS) 19: Oxidized (SS)</b>		66-96
16	<b>T27[72-96]S-S</b>	<b>2687.154</b>	<b>2687.192</b>	<b>1</b>	<b>-14.174</b>	<b>0</b>	<b>EAENITGCAEHCSLNENITVPDTK 9: Oxidized (SS) 13: Oxidized (SS)</b>		72-96
17	T7	1083.559	1083.573	1	-13.614	1	VNFYAWKR		97-104
18	T8	2542.2948	2541.3261	1	-15.18	0	R.MEVGQQAQAVEVWQGLALLSEAVLR.G	oxidation: 1	105-127
19	T9	2359.2197	2358.2332	1	-8.82	0	R.GQALLVNSSQPWEPLQLHVDK.A		128-148
20	T10	2942.326	2942.585	1	-87.853	1	GQALLVNSSQPWEPLQLHVDKAVSGLR		128-154
21	T11	803.4918	802.4913	1	-8.40	0	R.SLTLLR.A		155-161
22	T12	2034.0934	2033.0906	1	-2.15	1	R.ALGAQKEAISPPDAASAAPLR.T		162-182
23	T13	1465.7619	1464.7573	1	-1.78	0	K.EAISPPDAASAAPLR.T		168-182
24	T14	924.477	924.479	1	-1.237		TITADTFR		183-190
25	T15	1052.4865	1051.5662	1	-82.65	1	R.TITADTFRK.L		183-191
26	T28	3668.1462	3666.9821	1	42.76	6	R.TITADTFRKLFYVSNFLRGKLYTGEACR.T		183-213
27	T29	1314.6692	1313.7244	1	-47.53	1	K.LFRVYSNFLR.G		192-201
28	T16	898.4783	897.4709	1	0.23	0	R.VYSNFLR.G		195-201
29	T17	1083.559	1083.595	1	-33.131	1	VYSNFLRGK		195-203

\* Amino acids located before and after the dot are not in the peptide composition, these amino acids flank the identified sequence.