

Purification of RLIP76 from malignant and non-malignant cells<sup>a</sup>

Fraction	Total RLIP76 Activity (nmol/min)			Total Protein ( $\mu$ g)	Specific Activity (nmol/min/mg)		
	Basal	In presence of DNP-SG	In presence of DOX		Basal	In presence of DNP-SG	In presence of DOX
<b>HAVSMC</b>							
Detergent Solubilized Crude Membrane	2.94 $\pm$ 0.17	4.56 $\pm$ 0.18	4.06 $\pm$ 0.22	5873 $\pm$ 422	0.50	0.78	0.69
DNP-SG Affinity Purified RLIP76	0.90 $\pm$ 0.07	1.62 $\pm$ 0.21	1.51 $\pm$ 0.16	15.42 $\pm$ 1.24	59	105	97
<b>HUVEC</b>							
Detergent Solubilized Crude Membrane	3.38 $\pm$ 0.39	4.72 $\pm$ 0.36	4.36 $\pm$ 0.47	6042 $\pm$ 451	0.56	0.78	0.72
DNP-SG Affinity Purified RLIP76	0.76 $\pm$ 0.08	1.36 $\pm$ 0.16	1.28 $\pm$ 0.11	14.31 $\pm$ 1.26	53	95	89
<b>HepG2</b>							
Detergent Solubilized Crude Membrane	3.51 $\pm$ 0.38	5.42 $\pm$ 0.62	5.28 $\pm$ 0.59	5763 $\pm$ 529	0.61	0.94	0.92
DNP-SG Affinity Purified RLIP76	1.08 $\pm$ 0.12	1.98 $\pm$ 0.16	1.92 $\pm$ 0.21	17.41 $\pm$ 1.44	62	114	110
<b>H1618</b>							
Detergent Solubilized Crude Membrane	5.16 $\pm$ 0.39	8.36 $\pm$ 0.74	8.82 $\pm$ 0.79	6148 $\pm$ 322	0.84	1.36	1.44
DNP-SG Affinity Purified RLIP76	3.31 $\pm$ 0.25	6.16 $\pm$ 0.47	6.61 $\pm$ 0.39	32.42 $\pm$ 2.92	102	190	204

<b>H358</b>							
Detergent Solubilized Crude Membrane	8.84 ± 0.68	13.46 ± 0.92	14.52 ± 1.62	6378 ± 388	1.39	2.11	2.28
DNP-SG Affinity Purified RLIP76	6.06 ± 0.43	11.76 ± 0.86	12.66 ± 0.74	36.44 ± 2.86	166	323	348
<b>OVCAR-3</b>							
Detergent Solubilized Crude Membrane	11.64 ± 0.87	17.96 ± 1.22	18.81 ± 1.29	7882 ± 524	1.48	2.28	2.39
DNP-SG Affinity Purified RLIP76	6.72 ± 0.28	17.28 ± 1.17	18.62 ± 1.33	52.56 ± 3.44	128	329	354
<b>PC-3</b>							
Detergent Solubilized Crude Membrane	10.78 ± 0.68	16.48 ± 0.89	17.44 ± 1.19	7335 ± 398	1.47	2.25	2.38
DNP-SG Affinity Purified RLIP76	7.42 ± 0.53	15.64 ± 0.94	16.24 ± 0.76	46.22 ± 2.88	161	339	352
<b>MCF-7</b>							
Detergent Solubilized Crude Membrane	3.18 ± 0.31	4.76 ± 0.22	4.34 ± 0.29	6112 ± 622	0.52	0.78	0.71
DNP-SG Affinity Purified RLIP76	0.84 ± 0.11	1.48 ± 0.11	1.42 ± 0.12	14.82 ± 1.16	57	100	96
<b>B16 mouse melanoma</b>							
Detergent Solubilized Crude Membrane	16.26 ± 1.38	24.4 ± 1.66	25.44 ± 1.27	8846 ± 519	1.84	2.76	2.88
DNP-SG Affinity Purified RLIP76	8.76 ± 0.73	24.1 ± 1.32	25.04 ± 1.47	70.72 ± 5.66	124	341	354
<b>DG-1 human melanoma</b>							
Detergent Solubilized Crude Membrane	14.33 ± 1.82	21.82 ± 2.12	23.33 ± 1.92	8336 ± 655	1.72	2.62	2.8
DNP-SG Affinity Purified RLIP76	8.14 ± 1.11	20.15 ± 1.73	22.11 ± 1.55	62.64 ± 5.22	130	322	353

<b>HLMVEC</b>							
Detergent Solubilized Crude Membrane	2.66 ± 0.22	4.21 ± 0.28	4.03 ± 0.34	5996 ± 478	0.44	0.70	0.67
DNP-SG Affinity Purified RLIP76	0.94 ± 0.11	1.96 ± 0.24	1.82 ± 0.21	19.26 ± 2.18	49	101	94
<b>HLBEC</b>							
Detergent Solubilized Crude Membrane	3.31 ± 0.37	5.04 ± 0.51	4.82 ± 0.36	6088 ± 422	0.54	0.83	0.79
DNP-SG Affinity Purified RLIP76	1.16 ± 0.11	2.37 ± 0.18	2.18 ± 0.26	21.84 ± 1.91	53	108	100

<sup>a</sup>Purifications were performed with 100 x 10<sup>6</sup> cells in log phase growth (Singhal et al., Int. J. Oncol., 22: 365-75, 2003). Substrate (either DNP-SG or DOX) stimulated ATPase activity was determined in the presence of either 0.12 mM DNP-SG or 0.01 mM DOX, and ouabain (1 mM) and EGTA (2 mM) as detailed in the *Methods Section*. For basal ATPase activity, substrate was omitted from the reaction mixture. Each value represents mean ± SD (n=3).