MILLIPLEX® MAP Human Myokine Panel is an optimized, quantitative immunoassay that simultaneously measures 15 novel muscle-secreted factors

Skeletal muscle is actively involved in the synthesis and secretion of many proteins collectively termed "myokines."[1] Myokines can act in autocrine and/or paracrine manners to regulate skeletal muscle metabolism and muscle proliferation and differentiation (muscle growth), as shown in Figure 1.

In addition, these myokines can act as endocrine hormones in altering cell metabolism, endothelial function and tumor growth/retardation. These myokines are also involved in inflammatory responses in a wide variety of tissues, including heart, adipose tissue, breast, colon, liver, intestine, endothelium, bone and skeletal muscle.

Emerging evidence supports the fact that the anti-inflammatory, disease-fighting effects of exercise may be mediated by these myokines, and that myokine signaling pathways intersect with innate immunity, neurological signaling and insulin response[2,3].

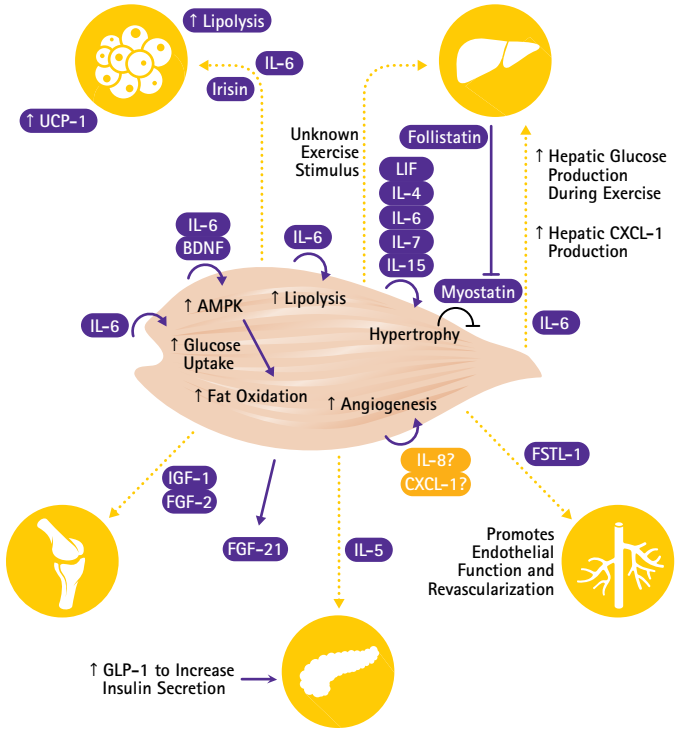

Figure 1.

Myokines, secreted by skeletal muscle, signal to diverse organs, including the liver, pancreas, bone and circulatory system. The precise mechanisms by which angiogenesis is upregulated are still being studied. Adapted from Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012 Apr 3; 8(8): 457 – 65.

To meet the increasing need to quantify myokines in preclinical and translational research models, Merck has developed the MILLIPLEX® MAP Human Myokine Panel. This 15-plex kit is designed for the simultaneous quantification of any or all of the following analytes in serum or plasma samples, which have the indicated biological functions:

- **APLN:** Apelin – The function of apelin depends on the tissue in which it is being expressed. Apelin regulates blood pressure in vascular tissue, heart contractions in cardiac tissue, food and water intake in the brain, and glucose uptake and insulin inhibition in the digestive tract.
- **BDNF:** Brain-derived neurotrophic factor – Associated with long-term memory, BDNF secretion increases in response to exercise.
- **EPO:** Erythropoeitin – Because it stimulates red blood cell production, it may be administered to athletes to increase oxygen supply to the body, ostensibly improving performance.
- **FABP3:** Fatty acid-binding protein 3 – This myokine is produced by heart muscle tissue and regulates cardiac uptake of long-chain fatty acids. It is a reliable and early biomarker of myocardial infarction and acute coronary events.
- **FGF21:** Fibroblast growth factor 21 – FGF21 is activated by Akt and is a metabolism-regulating hormone and myokine.
- **Irisin:** The cleaved and secreted portion of fibronectin type III domain-containing protein 5 (FNDC5) – The once-debated function of irisin has now been settled by a recent proteomics study using tandem mass spectrometry[4]. Irisin levels increase in response to exercise and may mediate the browning of white fat.
- **FSTL1:** Follistatin-related protein 1 – Regulated by nitric oxide signaling, FSTL1 induces growth of new blood vessels in muscle and protects cardiac myocyte from ischemic-induced apoptosis.
- **CX3CL1:** Fractalkine – CX3CL1 is a chemokine protein expressed on monocytes, natural killer cells, T cells, and smooth muscle cells. It has recently been associated with obesity, insulin resistance and Type 2 Diabetes.
- **IL-6:** Interleukin 6 – IL-6 is, in a way, a "master myokine," in that it regulates multiple, divergent processes in response to exercise, ranging from glucose production, fat oxidation and lipolysis. IL-6 also regulates the immune response to exercise and triggers anti-inflammatory signaling.
- **IL-15:** Interleukin 15 – IL-1 5 regulates T and NK cell activation and proliferation. As a myokine, it significantly reduces visceral fat.
- **LIF:** Leukemia inhibitory factor – This protein inhibits differentiation and may activate cardiac and muscle stem cells in response to exercise.
- **GDF8:** Growth differentiation factor 8 (Myostatin) – In a feedback loop, myostatin inhibits the growth of muscle cells and blocks the differentiation of stem cells toward muscle lineages.
- **OSM:** Oncostatin M – Oncostatin is in the IL-6 family of cytokines and likely shares functions of IL-6, particularly in the regulation of inflammation.
- **OSTN:** Osteocrin (Musclin) – The main role of musclin is in the regulation of glucose metabolism.
- **SPARC:** Secreted protein acidic and rich in cysteine (Osteonectin) – SPARC is a myokine that blocks tumor progression in the colon by inducing apoptosis, in response to exercise.

Materials and Methods

Multiplex assays using the standards included in the assay kit, as well as serum and plasma samples, were conducted using the MILLIPLEX® MAP Human Myokine Magnetic Bead Panel (Cat. No. HMYOMAG-56K), following the instructions in the included protocol. Capture antibody-coated beads were incubated with samples in order to immobilize the analyte. The biotinylated detection antibody was added; this detection antibody binds to the analyte to form a sandwich. Streptavidin-phycoerythrin (SA-PE) dye was added so it could bind to the sandwich and emit fluorescent light.

Results

Using standards for each analyte serially diluted in serum matrix, standard curves were prepared to determine the assay response (measured in Mean Fluorescence Intensity [MFI] with respect to analyte concentration). The standard curves established that the assay generated linear MFI response over 3 to 5 orders of magnitude for all analytes (Figure 2). No significant cross-reactivity was seen within the panel (Table 1).

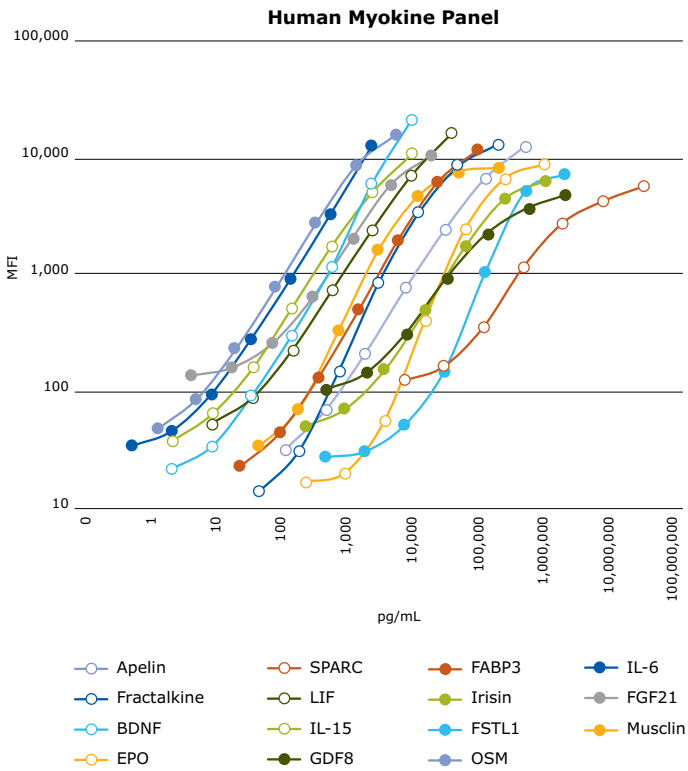

Figure 2.

Using standards for each analyte serially diluted in the validated serum matrix, standard curves were prepared to determine the assay response with respect to analyte concentration. The standard curves established linearity of the assay over three to four orders of magnitude for all analytes. The concentrations of analyte were measured in Mean Fluorescence Intensity (MFI).

Cross-Reactivity of Myokine Multiplexed Assays

	MFI	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
Frac	std 4	21	1108	17	15	109	43	27	91	15	45	25	32	29	122	27
		20	1115	16	16	113	43	27	100	15	44	26	35	30	133	27
	std 5	20	3985	17	16	113	43	30	95	14	44	27	33	31	135	26
		20	4039	17	15	116	43	30	97	15	44	26	34	30	135	27
	std 6	22	8821	18	17	115	46	29	91	17	47	24	36	30	130	27
		20	8775	16	17	119	45	29	94	15	45	27	34	30	132	25
	std 7	21	13807	18	16	121	47	30	97	17	47	28	36	30	133	27
		23	13635	18	16	120	44	30	96	17	49	28	37	32	136	27
BDNF	std 4	17z	9	307	13	112	40	26	84	13	35	23	36	26	117	22
		16	12	347	15	119	43	24	84	13	37	22	32	26	111	21
	std 5	15	12	1419	14	114	43	26	78	13	38	21	37	30	117	19
		18	9	1409	12	112	41	24	86	10	36	23	36	27	119	21
	std 6	18	9	7273	15	123	41	25	87	13	40	23	34	26	124	21
		18	11	8366	15	124	42	27	88	14	36	23	35	29	119	22
	std 7	17	10	21571	15	121	39	28	87	10	34	19	33	28	114	24
		18	10	21264	14	118	41	26	80	12	35	25	34	27	108	22
EPO	std 4	21	11	14	605	104	42	28	89	14	45	26	31	30	121	25
		20	13	16	582	111	43	27	96	16	45	25	32	28	137	25
	std 5	19	12	17	2911	114	44	28	92	16	45	26	33	30	134	26
		20	12	18	3016	114	44	28	90	15	41	27	31	29	125	26
	std 6	19	12	23	7275	121	47	31	102	16	50	26	32	31	139	26
		20	12	20	7080	120	48	28	99	17	48	26	33	30	136	28
	std 7	21	12	43	8686	117	43	27	97	15	44	26	33	29	127	27
		19	12	37	8809	118	44	29	92	15	46	25	33	29	130	27
SPARC	std 4	18	12	17	16	1377	41	26	92	14	44	26	31	28	123	26
		19	11	16	15	1359	42	29	94	16	47	22	31	28	133	25
	std 5	21	12	16	15	3061	41	29	91	14	44	24	31	29	127	26
		19	11	14	16	3031	42	27	89	13	42	25	32	28	125	25
	std 6	18	12	16	16	4390	44	28	87	15	42	24	32	29	124	25
		19	12	17	16	4420	42	28	87	14	45	25	31	29	123	26
	std 7	20	11	16	17	5749	41	27	89	15	47	25	32	31	125	25
		19	11	17	16	5680	42	27	95	15	47	25	31	29	125	27
LIF	std 4	18	12	15	16	100	1296	26	80	16	43	22	29	28	109	26
		19	12	14	16	112	1360	27	91	16	45	25	31	29	125	26
	std 5	20	11	15	15	108	4085	27	93	16	46	24	31	29	125	25
		19	12	15	16	111	4059	27	90	15	44	25	32	28	127	26
	std 6	22	11	16	16	114	9555	27	90	14	42	24	32	28	117	25
		22	12	15	14	116	9624	28	94	14	46	24	33	28	127	25
	std 7	24	12	18	16	125	18040	28	89	15	44	25	36	28	128	25
		26	12	19	17	116	18661	27	92	15	47	25	35	30	134	27
IL-15	std 4	19	10	14	15	102	38	590	90	14	43	25	30	29	124	26
		19	11	16	16	112	41	572	101	15	44	24	31	31	132	25
	std 5	18	12	15	16	112	41	2036	100	14	45	25	31	28	133	25
		19	11	15	16	108	40	1970	94	14	44	22	30	28	134	26
	std 6	20	11	16	15	110	42	4888	95	15	43	25	31	29	134	25
		18	11	16	17	107	42	5039	94	13	43	24	31	29	133	25
	std 7	19	12	16	15	113	51	10834	98	15	45	25	31	28	136	25
		20	12	16	16	108	51	10730	97	14	45	24	34	30	135	25
GDF8	std 4	19	11	14	15	100	39	26	425	14	43	24	30	27	115	25
		19	12	17	15	106	38	25	421	13	46	25	31	30	125	25
	std 5	19	11	15	15	106	39	25	1269	13	45	23	31	28	127	24
		18	11	15	15	104	39	25	1199	13	48	25	31	27	120	24
	std 6	20	12	15	14	102	38	27	2605	14	60	23	31	30	124	25
		19	11	15	16	107	38	29	2693	14	63	23	31	29	126	25
	std 7	20	11	17	16	114	41	25	3847	14	126	23	32	29	123	25
		18	12	18	15	105	41	27	3897	16	125	23	30	28	125	26

Table 1.

Minor cross-reactivity (<5%) was observed between the musclin standard and all other analytes' antibodies. In addition, the musclin sample values fall in the low end of standard curve. Considering the musclin sample values fall in the low end of the standard curve, this amount of cross-reactivity would not be expected to affect the assay accuracy.

Cross-Reactivity of Myokine Multiplexed Assays (continued)

	MFI	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
FABP3	std 4	18	13	19	16	92	48	32	105	568	49	28	34	34	150	28
		22	17	20	20	97	51	31	107	591	46	28	32	31	147	28
	std 5	20	14	19	17	98	49	32	104	2204	48	29	32	31	144	28
		22	15	21	17	103	47	33	110	2337	50	29	32	33	150	26
	std 6	21	15	20	17	104	52	32	110	6903	50	31	36	34	150	32
		19	16	20	18	101	52	35	112	6886	53	31	34	35	153	29
	std 7	23	14	20	21	108	51	30	113	12255	51	31	36	34	156	28
		20	15	20	18	104	51	28	108	12404	52	29	33	31	146	28
Irisin	std 4	26	20	22	22	108	56	36	110	22	709	32	37	39	159	36
		30	19	24	22	104	52	39	111	20	733	39	40	46	163	37
	std 5	25	16	23	27	101	56	37	124	22	2360	38	41	43	161	35
		28	19	26	22	114	56	34	123	24	2339	33	40	40	165	33
	std 6	27	16	24	20	111	56	35	112	22	5265	35	40	38	150	34
		25	18	27	21	115	55	36	114	23	5304	35	39	39	166	35
	std 7	28	18	24	22	113	55	38	107	25	6802	35	40	40	161	35
		26	15	24	24	115	54	35	111	21	6814	36	37	43	159	36
FSTL1	std 4	27	19	24	22	101	54	34	109	19	56	226	36	40	146	30
		26	16	27	20	111	59	37	117	19	58	238	37	37	169	33
	std 5	25	18	25	23	118	51	33	116	19	63	1384	37	34	163	35
		26	18	25	23	112	54	34	111	21	59	1377	38	39	157	36
	std 6	26	16	22	20	104	58	37	112	21	67	5904	38	42	155	35
		26	17	24	21	116	51	37	111	22	70	6156	38	44	166	34
	std 7	26	18	26	20	115	58	35	111	24	105	8090	39	37	174	33
		26	21	26	22	124	55	33	110	26	105	8025	40	38	177	36
OSM	std 4	23	20	22	21	111	56	36	106	22	54	31	1201	39	143	33
		20	18	24	22	107	53	35	108	23	55	32	1192	39	161	31
	std 5	24	17	24	21	106	54	40	103	21	54	31	3857	40	154	33
		25	19	24	22	110	53	37	112	24	54	32	3711	39	155	33
	std 6	25	18	21	23	106	51	34	103	23	56	31	10305	41	156	34
		27	19	23	21	111	55	35	113	26	58	30	10428	40	159	33
	std 7	26	19	23	24	122	53	39	104	23	54	31	17246	39	154	30
		26	19	22	25	105	55	36	120	22	57	35	17557	43	160	34
IL-6	std 4	31	17	20	23	101	52	36	121	21	56	35	40	403	169	33
		26	18	22	20	110	55	32	115	22	52	35	38	381	166	35
	std 5	25	16	25	22	108	52	38	128	23	57	32	40	1314	175	33
		26	18	24	23	112	55	38	130	22	57	31	44	1325	178	36
	std 6	26	16	20	21	107	50	38	108	24	52	31	40	4245	155	33
		22	21	25	23	117	53	33	118	21	56	35	41	4445	159	36
	std 7	26	17	24	24	113	51	41	105	24	52	34	43	15480	161	32
		24	17	26	24	114	53	38	113	22	55	32	44	15857	161	33
FGF21	std 4	25	17	22	23	107	52	34	109	23	52	32	39	38	1298	34
		24	18	22	21	107	51	35	120	24	56	32	35	37	1251	33
	std 5	23	16	23	25	111	51	37	117	20	59	31	42	38	4078	35
		26	18	23	23	108	53	37	114	20	55	32	39	40	3879	35
	std 6	26	23	21	26	111	52	35	114	23	63	32	46	40	9184	37
		24	21	25	26	108	55	36	116	25	63	33	40	41	8977	40
	std 7	37	37	34	32	120	58	39	130	30	97	45	64	41	13589	52
		34	37	33	29	126	63	41	123	29	102	40	68	40	13740	56
Musclin	std 4	34	18	24	24	108	63	34	112	27	58	35	48	42	157	2097
		30	20	22	26	110	67	40	113	25	56	39	43	36	161	2056
	std 5	53	27	42	42	129	127	42	146	42	83	63	78	41	196	5795
		52	34	50	42	134	125	43	145	40	85	56	85	45	207	5751
	std 6	180	97	200	138	278	392	75	286	154	276	184	306	64	352	8683
		193	92	206	147	283	407	80	300	155	291	211	316	73	374	8251
	std 7	676	353	719	514	801	1332	212	810	577	923	712	1176	138	1001	9107
		754	393	824	552	880	1487	235	892	605	1036	791	1223	158	1082	9132
	%	**1.4**	**0.7**	**3.0**	**1.6**	**0.0**	**2.8**	**0.5**	**1.1**	**1.5**	**2.6**	**4.4**	**1.9**	**0.5**	**2.2**	

Table 1 (continued).

Minor cross-reactivity (<5%) was observed between the musclin standard and all other analytes' antibodies. In addition, the musclin sample values fall in the low end of standard curve. Considering the musclin sample values fall in the low end of the standard curve, this amount of cross-reactivity would not be expected to affect the assay accuracy.

Most of the antibodies in this human myokine panel can recognize monkey samples, except Fractalkine, LIF and IL-6 (Table 2). Apelin cross-reacts with mouse, dog, rabbit and minipig. BDNF cross-reacts with mouse and rabbit. EPO cross-reacts with dog and rabbit. IL-15 cross-reacts with horse. Myostatin cross-reacts with dog, minipig and horse. FABP3 cross-reacts with rat, rabbit, minipig and horse. FSTL1 cross-reacts with mouse, rabbit, minipig and horse. OSM cross-reacts with dog and minipig. FGF21 cross-reacts with dog, minipig and horse. Note that only limited amount of normal samples were tested in this study, and we could not rule out the possible cross-reactivity in other normal or disease samples.

Myokine Concentration [pg/mL]

	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
Human Exercise Sample 1	198	396	10844	1847	307150	48	28		40040	10652	16352	126	6143	5996	90
Human Exercise Sample 2	45	35	8238		289560				12736	2913	11651	16	187	2657	76
Human Exercise Sample 3	64	192	95	6779	173870		11		1258	10292	6379		91		104
Human Exercise Sample 4	254	137	15964	1152	271440			18312	6835	1539	9705	9	10	434	157
Monkey 1	122		172	8475	262260		14	1136	10811		2100	7		404	246
Monkey 2	170		3180	2006	172540				9451	768				145	2678
Monkey 3	163		2335	28661	359810		25		6345		3308	14		588	1630
Monkey 4	290		1061	7003	210100		20		10343		6088			367	75
Rat 1			4800						2477						
Rat 2			269						1971					152	
Rat 3			627						2948						
Rat 4			4725						2732						
Mouse 1			11						8						
Mouse 2	3715		208			157			50		7102				
Mouse 3	1615		82			61			36		8665				
Mouse 4									7	6289					
Dog 1				862				11409	163			36		449	
Dog 2	1244		100		7110				36					239	
Dog 3				443				2890	6			31		171	
Dog 4								12413	8	768		33		394	
Rabbit 1	876		44		6110				317		17725				
Rabbit 2	672		36						232		12108				
Rabbit 3	906		33	416					229		14563				
Rabbit 4	918		37						206		15646				
Minpig 1								9578	8	1539	21471			95	
Minpig 2								9356	8	706	44759	21		104	
Minpig 3	118			735				4442	102			19			
Minpig 4	696	797		1290	22700	94	17	478343	529	1918	18559	345		2066	
Horse 1							19	10505	451		6814			115	
Horse 2							33	7474	218		7747		11		
Horse 3							22	11606	491		7960			134	
Horse 4							18	1043	165		3598				

Table 2.

Samples, taken from various species, show not all myokines are expressed to the same degree in all subjects or tissues.

	Apelin	Frac-talkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
pg/mL															
std 1	122	49	2	244	7324	10	2	488	24	244	488	1	1	5	49
std 2	488	195	10	977	29297	39	10	1953	98	977	1953	6	2	20	195
std 3	1953	781	39	3906	117188	156	39	7813	391	3906	7813	23	9	78	781
std 4	7813	3125	156	15625	468750	625	156	31250	1563	15625	31250	94	38	313	3125
std 5	31250	12500	625	62500	1875000	2500	625	125000	6250	62500	125000	375	150	1250	12500
std 6	125000	50000	2500	250000	7500000	10000	2500	500000	25000	250000	500000	1500	600	5000	50000
std 7	500000	200000	10000	1000000	30000000	40000	10000	2000000	100000	1000000	2000000	6000	2400	20000	200000
MFI															
std 1	33	14	22	17	124	54	38	104	23	51	28	48	35	137	34
std 2	69	31	35	21	163	89	65	143	44	71	31	88	46	161	71
std 3	212	150	94	58	354	227	157	310	133	155	53	243	95	258	333
std 4	750	849	307	411	1,154	736	524	906	495	493	155	806	282	629	1,625
std 5	2420	3328	1175	2431	2770	2401	1767	2169	1999	1717	1029	2839	930	2029	4693
std 6	6581	8392	5990	6602	4213	7084	4981	3603	6238	4441	5059	8730	3321	5997	7515
std 7	12355	12910	21111	8705	5622	16056	10651	4744	11610	6275	7170	15794	12158	10415	8098
Sensitivity	**63.9**	**40.4**	**8.4**	**814.6**	**7481.1**	**4.1**	**4.9**	**258.6**	**9.7**	**281.2**	**736.7**	**5.7**	**0.9**	**14.1**	**59.2**
Intra-Assay CV% QC (10 assays)	2.1	2.7	3.6	1.6	2.5	2.2	1.9	2.8	2.6	3.9	3.1	5.6	4.2	4.1	7.2
Inter-Assay CV% QC (10 assays)	10.7	7.9	14.2	5.9	15.3	6	9.4	7.7	9	8.8	7.6	9.5	13	10	11.9

Table 3.

Assay precision, as determined by measuring signals from included standards.

Known amounts of each analyte (points 3, 4, 5 on the standard curves) were spiked into normal human serum/plasma samples. Percent recovery was calculated using the following equation: Calculated concentration of analyte / Expected concentration *100 (Table 4).

Spike and Recovery in serum [n=5] and plasma [n=4] samples

	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
Average															
+std 3	100	96	105	71	90	95	109	95	97	105	119	84	100	82	106
+std 4	92	104	113	77	90	101	105	128	93	115	114	77	130	92	109
+std 5	85	94	135	72	75	101	99	150	90	99	97	72	155	90	156
Average	**93**	**98**	**118**	**74**	**85**	**99**	**104**	**124**	**93**	**106**	**110**	**77**	**129**	**88**	**124**

Table 4.

Recovery of all spiked samples was between 70% and 130%.

Next, linearity tests were performed to determine the extent to which the dose-response of each analyte was linear in a particular diluent (Table 5). Calculations rely on dividing the Observed Values by the Expected Values. Unfortunately, some analytes were barely detectable in the human sepsis samples we tested. We were, therefore, compelled to spike known amounts of protein standards into normal serum/plasma samples and test the dilution linearity. The linearity range was 70% to 130% (Table 4). The kit components were stable over a wide range of temperatures. Storing the kit at 37°C for 7 days did not significantly alter the assay performance (data not shown).

Average Dilution Linearity in Human Serum/Plasma Samples [n=10 samples]

	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
1:4 dil			64	79	139		94	110	89		67	99	79	114	
1:8 dil			49	54	150		98	111	86		79	89	66	124	
1:16 dil			93	79	120		118	89	95		109	84	98	93	
Average			**69**	**71**	**136**		**103**	**103**	**90**		**85**	**90**	**81**	**110**	

Table 5.

Average dilution linearity in human serum/plasma samples.

Average Dilution Linearity in Spiked Serum/Plasma Samples [n=10 samples] (continued)

	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
1:4 dil	111	85	58	105	100	97	95	74	92	106	73	106	77	91	109
1:8 dil	123	84	48	108	98	91	94	63	87	105	70	110	66	84	111
1:16 dil	129	84	42	108	87	90	94	61	83	100	64	114	61	75	116
Average	**121**	**84**	**49**	**107**	**95**	**93**	**94**	**66**	**87**	**104**	**69**	**110**	**68**	**83**	**112**

Table 5 (continued).

Average dilution linearity in human serum/plasma samples.

To test the consistency and accuracy between MILLIPLEX® assays, we compared the quantitation of analytes existing in both this human myokine panel and other MILLIPLEX® panels. All the standard curves showed overlapping response and analyte concentrations calculated in biological samples correlated very well.

For example, FGF21 and FABP3 sample values were measured using both MILLIPLEX® Human Myokine Panel, Human Liver Protein Panel and Human CVD1 Panel. Standard curve created using standards of purified FGF21, and FABP3 from two panels showed overlapping assay response and linear range of the assays. Assay correlation was excellent, with slope and R value approaching unity (Figure 3).

In addition to FGF21 and FABP3, we have compared the same analytes in this human myokine panel with other MILLIPLEX® panels. All the standard curves showed overlapping responses and analyte concentrations that correlated very well with biological samples (data not shown).

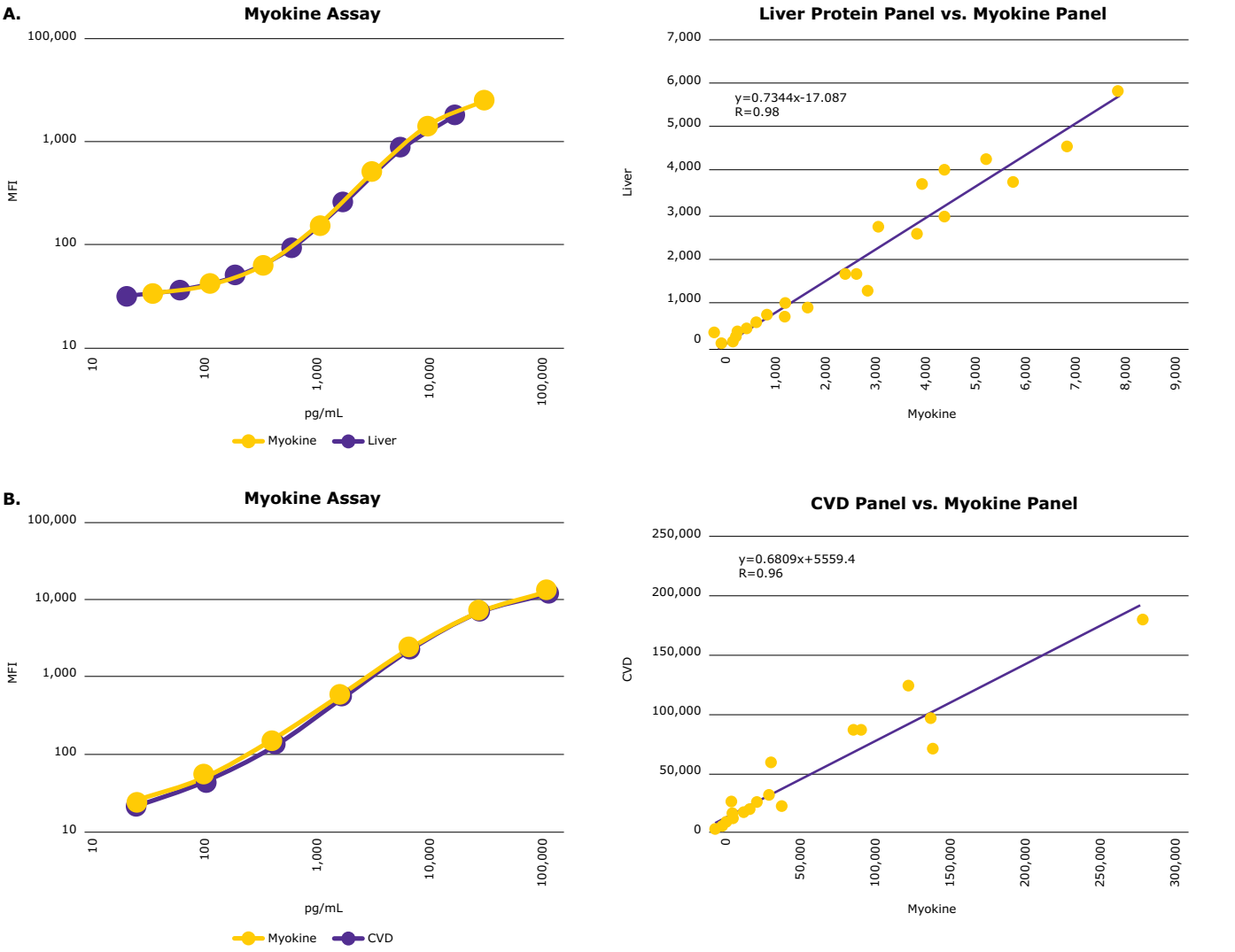

Figure 3.

Comparison of the MILLIPLEX® MAP Human Myokine Panel with the Liver Panel and CVD Panel for the measurement of FGF21 (top) and FABP3 (bottom) respectively.

Finally, biological qualification of the assay panel was performed using serum/plasma samples from healthy subjects and from those with sepsis. As expected, 15 novel myokines were simultaneously quantified by the assay. FABP3, Irisin, FSTL1, OSM, IL-6, FGF21 and Musclin were significantly upregulated in sepsis samples (Table 6).

Normal Sample Summary Data [pg/mL]

	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
N	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Average	228	101	9548	1239	425343	10	3	8625	1943	495	6491	13	3	134	150
Min	0	0	350	0	169720	0	0	0	740	0	0	0	0	0	0
Max	420	260	20480	3460	739890	30	20	40110	3110	2370	17170	37	9	570	790
% detectable	**88**	**88**	**100**	**63**	**100**	**50**	**13**	**38**	**100**	**38**	**88**	**75**	**88**	**63**	**50**

Sepsis Sample Summary Data [pg/mL]

	Apelin	Fractalkine	BDNF	EPO	SPARC	LIF	IL-15	Myostatin	FABP3	Irisin	FSTL1	OSM	IL-6	FGF21	Musclin
N	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Average	122	78	9280	1298	544958	6	7	3777	38972	1905	14632	56	643	2016	174
Min	0	0	110	0	204800	0	0	0	240	0	0	0	0	0	0
Max	460	410	20180	12520	1258000	80	60	31600	249140	9060	74170	317	8679	12400	88...0
% detectable	**69**	**69**	**100**	**41**	**100**	**19**	**25**	**28**	**100**	**60**	**84**	**97**	**81**	**75**	**59**

Table 6.

Comparison of biomarkers expressed in normal and sepsis serum/plasma samples. FABP3, Irisin, FSTL1, OSM, IL-6, FGF21 and Musclin were shown to be upregulated in sepsis samples.

Conclusion

The MILLIPLEX® MAP Human Myokine Panel is sensitive, accurate and reproducible. The sample values generated with this new panel are consistent with previous panels that include some of the same analytes. This panel provides an ideal immunoassay for diverse research areas, including metabolic, neuromuscular and idiopathic myopathy diseases.

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